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CONTENTS—PART 3

*12,361 volunteer abstracts, 16 symposia abstracts, 20 history of neuroscience abstracts, and 41 teaching of neuroscience abstracts.

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

Recently Council approved a revised version of the Society's Policy on the Use of Animals in Neuroscience Research and adopted a new policy on the use of human subjects in neuroscience research. The revised version of the animal policy, drafted by the Committee on Animals in Research, in conjunction with the editor-in-chief of *The Journal of Neuroscience* and the chair of the Publications Committee, is printed below. The amendment is contained in the "Recommended References" section. The Policy on the Use of Human Subjects in Neuroscience Research is printed after the animal policy.

Policy on the Use of Animals in Neuroscience Research

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

Introduction

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

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

General Policy

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

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

Local Committee Review

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

component of the Society Policy. Assistance in developing appropriate animal care and use procedures and establishing a local review committee can be obtained from the documents listed below and from the Society.

Other Laws, Regulations, and Policies

In addition to complying with the policy described above, Regular Members (i.e., North American residents) of the Society must also adhere to all relevant national, state, or local laws and/or regulations that govern their use of animals in neuroscience research. Thus, U.S. members must observe the U.S. Animal Welfare Act (as amended in 1985) and its implementing regulations from the U.S. Department of Agriculture. Canadian members must abide by the *Guide to the Care and Use of Experimental Animals*, and members in Mexico must comply with the *Reglamento de la Ley General de Salud en Materia de Investigacion para la Salud of the Secretaria de Salud* (published on Jan. 6, 1987). Similarly, in addition to complying with the laws and regulations of their home countries, Foreign Members of the Society should adhere to the official Society Policy outlined here.

Recommended References

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

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

Guide for the Care and Use of Laboratory Animals. NIH Publication No. 85-23 (revised 1985). NIH, Bldg. 14A, Rm. 100, 9000 Rockville Pike, Bethesda, MD 20892.

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

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

OPRR Public Health Service Policy on Humane Care and Use of Laboratory Animals (revised Sept. 1986). Office for Protection from Research Risks, NIH, 6100 Executive Blvd., Suite 3B01-MSC 7509, Rockville, MD 20892-7509.

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

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

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

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

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

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

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

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

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

POLICY ON THE USE OF HUMAN SUBJECTS IN NEUROSCIENCE RESEARCH

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

Recommended References

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

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

POLICY ON ETHICS

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

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

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

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

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

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

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

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

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

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

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

CHRONOLOGICAL LIST OF SESSIONS

(See page xix for Thematic List of Sessions)

MONDAY, NOV. 13

TUESDAY, NOV. 14

WEDNESDAY, NOV. 15

Session Number & Title — Page

THURSDAY, NOV. 16

Symposia—8:00 a.m.

Slide Sessions—8:00 a.m.

Poster Sessions—8:00 a.m.

THEMATIC LIST OF SESSIONS

(Includes slide and poster sessions and symposia only.)

Session Number	Session Title	Type	Day and Time Sun.	Mon.	Tue.	Wed.	Thu.
THEME A: DEVELOPMENT AND REGENERATION							
614.	Aging processes: anatomy	Poster				Wed AM	
236.	Aging processes: calcium	Poster		Mon AM			
797.	Aging processes: molecular characteristics	Poster					Thu AM
237.	Aging processes: physiology	Poster		Mon AM			
132.	Assisted axonal regeneration	Poster	Sun PM				
123.	Axon guidance mechanisms and pathways I	Poster	Sun PM				
124.	Axon guidance mechanisms and pathways II	Poster	Sun PM				
222.	Axon guidance mechanisms and pathways III	Poster		Mon AM			
322.	Axon guidance mechanisms and pathways IV	Poster		Mon PM			
406.	Axon guidance mechanisms and pathways V	Slide			Tue AM		
513.	Axon guidance mechanisms and pathways VI	Poster			Tue PM		
594.	Axon guidance mechanisms and pathways VII	Slide				Wed AM	
506.	Axonal regeneration I	Slide			Tue PM		
612.	Axonal regeneration II	Poster				Wed AM	
707.	Axonal regeneration III	Poster				Wed PM	
21.	Cell differentiation and migration I	Poster	Sun AM				
318.	Cell differentiation and migration II	Poster		Mon PM			
319.	Cell differentiation and migration III	Poster		Mon PM			
414.	Cell differentiation and migration IV	Poster			Tue AM		
415.	Cell differentiation and migration V	Poster			Tue AM		
601.	Cell differentiation and migration VI	Poster				Wed AM	
602.	Cell differentiation and migration VII	Poster				Wed AM	
785.	Cell differentiation and migration VIII	Slide					Thu AM
220.	Cell lineage and determination I	Poster		Mon AM			
315.	Cell lineage and determination II	Slide		Mon PM			
511.	Cell lineage and determination III	Poster			Tue PM		
697.	Cell lineage and determination IV	Poster				Wed PM	
26.	Cerebral cortex and limbic system I	Poster	Sun AM				
129.	Cerebral cortex and limbic system II	Poster	Sun PM				
593.	Cerebral cortex and limbic system III	Slide				Wed AM	
794.	Cerebral cortex and limbic system IV	Poster					Thu AM
328.	Development of tectum and optic nerve	Poster		Mon PM			
509.	Development of visual system I	Slide			Tue PM		
591.	Development of visual system II	Slide				Wed AM	
517.	Development of visual thalamus	Poster			Tue PM		
681.	**Developmental Determinants of Retinal Ganglion Cells**	SYMP				**Wed PM**	
687.	Formation and specificity of synapses I	Slide				Wed PM	
701.	Formation and specificity of synapses II	Poster				Wed PM	
700.	Formation and specificity of synapses: agrin	Poster				Wed PM	
324.	Formation and specificity of synapses: cell-cell interactions	Poster		Mon PM			
323.	Formation and specificity of synapses: molecules of synapses	Poster		Mon PM			
493.	**GDNF: A New Neurotrophic Factor with Multiple Roles**	SYMP			**Tue PM**		
122.	Genesis of neurons and glia I	Poster	Sun PM				
317.	Genesis of neurons and glia II	Poster		Mon PM			
510.	Genesis of neurons and glia III	Poster			Tue PM		
595.	Genesis of neurons and glia IV	Slide				Wed AM	
600.	Genesis of neurons and glia V	Poster				Wed AM	

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
25.	Glia and other non-neuronal cells I	Poster	Sun AM				
128.	Glia and other non-neuronal cells II	Poster	Sun PM				
231.	Glia and other non-neuronal cells III	Poster		Mon AM			
327.	Glia and other non-neuronal cells IV	Poster		Mon PM			
425.	Glia and other non-neuronal cells V	Poster			Tue AM		
24.	Hormones and development I	Poster	Sun AM				
423.	Hormones and development II	Poster			Tue AM		
791.	Hormones and development III	Poster					Thu AM
426.	Molecular changes after lesions	Poster			Tue AM		
705.	Motor systems	Poster				Wed PM	
203.	**Neural Progenitor Cells and CNS Lineage Development**	SYMP		**Mon AM**			
7.	Neuronal death I	Slide	Sun AM				
127.	Neuronal death II	Poster	Sun PM				
230.	Neuronal death III	Poster		Mon AM			
326.	Neuronal death IV	Poster		Mon PM			
424.	Neuronal death V	Poster			Tue AM		
516.	Neuronal death VI	Poster			Tue PM		
610.	Neuronal death VII	Poster				Wed AM	
704.	Neuronal death VIII	Poster				Wed PM	
793.	Neuronal death IX	Poster					Thu AM
223.	Neurotransmitter systems and channels I	Poster		Mon AM			
514.	Neurotransmitter systems and channels II	Poster			Tue PM		
702.	Neurotransmitter systems and channels III	Poster				Wed PM	
119.	Neurotrophic factors: biologic effects I	Slide	Sun PM				
224.	Neurotrophic factors: biologic effects II	Poster		Mon AM			
225.	Neurotrophic factors: biologic effects III	Poster		Mon AM			
226.	Neurotrophic factors: biologic effects IV	Poster		Mon AM			
227.	Neurotrophic factors: biologic effects V	Poster		Mon AM			
418.	Neurotrophic factors: biologic effects VI	Poster			Tue AM		
419.	Neurotrophic factors: biologic effects VII	Poster			Tue AM		
420.	Neurotrophic factors: biologic effects VIII	Poster			Tue AM		
421.	Neurotrophic factors: biologic effects IX	Poster			Tue AM		
605.	Neurotrophic factors: biologic effects X	Poster				Wed AM	
606.	Neurotrophic factors: biologic effects XI	Poster				Wed AM	
607.	Neurotrophic factors: biologic effects XII	Poster				Wed AM	
608.	Neurotrophic factors: biologic effects XIII	Poster				Wed AM	
22.	Neurotrophic factors: expression and regulation I	Poster	Sun AM				
23.	Neurotrophic factors: expression and regulation II	Poster	Sun AM				
125.	Neurotrophic factors: expression and regulation III	Poster	Sun PM				
126.	Neurotrophic factors: expression and regulation IV	Poster	Sun PM				
417.	Neurotrophic factors: expression and regulation V	Poster			Tue AM		
604.	Neurotrophic factors: expression and regulation VI	Poster				Wed AM	
790.	Neurotrophic factors: expression and regulation VII	Poster					Thu AM
325.	Neurotrophic factors: receptors and cellular mechanisms I	Poster		Mon PM			
422.	Neurotrophic factors: receptors and cellular mechanisms II	Poster			Tue AM		
515.	Neurotrophic factors: receptors and cellular mechanisms III	Poster			Tue PM		
609.	Neurotrophic factors: receptors and cellular mechanisms IV	Poster				Wed AM	
780.	Neurotrophic factors: receptors and cellular mechanisms V	Slide					Thu AM
703.	Neurotrophic factors: receptors and cellular mechanisms—miscellaneous	Poster				Wed PM	
792.	Nutritional and prenatal factors	Poster					Thu AM
130.	Optic nerve regeneration	Poster	Sun PM				
228.	Other factors and trophic agents I	Poster		Mon AM			

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
229.	Other factors and trophic agents II	Poster		Mon AM			
118.	Pattern formation, compartments and boundaries I	Slide	Sun PM				
416.	Pattern formation, compartments and boundaries III	Poster			Tue AM		
603.	Pattern formation, compartments and boundaries IV	Poster				Wed AM	
13.	Process outgrowth, growth cones, and sprouting I	Slide	Sun AM				
221.	Process outgrowth, growth cones, and sprouting II	Poster		Mon AM			
321.	Process outgrowth, growth cones, and sprouting III	Poster		Mon PM			
512.	Process outgrowth, growth cones, and sprouting IV	Poster			Tue PM		
698.	Process outgrowth, growth cones, and sprouting V	Poster				Wed PM	
699.	Process outgrowth, growth cones, and sprouting VI	Poster				Wed PM	
789.	Process outgrowth, growth cones, and sprouting VII	Poster					Thu AM
131.	Regeneration of nervous systems	Poster	Sun PM				
611.	Retinal development	Poster				Wed AM	
233.	Sensory systems: activity-dependent mechanisms of development	Poster		Mon AM			
232.	Sensory systems: central and peripheral responses to nerve injury	Poster		Mon AM			
235.	Sensory systems: olfactory, gustatory, and acoustico-vestibular development	Poster		Mon AM			
234.	Sensory systems: somatosensory development	Poster		Mon AM			
329.	Transplant-assisted axonal regeneration	Poster		Mon PM			
330.	Transplantation I	Poster		Mon PM			
691.	Transplantation II	Slide				Wed PM	
796.	Transplantation: dissociated cells	Poster					Thu AM
518.	Transplantation: evidence of function	Poster			Tue PM		
613.	Transplantation: growth factors	Poster				Wed AM	
133.	Transplantation: Parkinson's disease—related	Poster	Sun PM				
706.	Visual cortical development I	Poster				Wed PM	
795.	Visual cortical development II	Poster					Thu AM
THEME B: CELL BIOLOGY							
331.	Blood-brain barrier: function	Poster		Mon PM			
688.	Blood-brain barrier: other	Slide				Wed PM	
429.	Blood-brain barrier: pathology and disease	Poster			Tue AM		
27.	Cytoskeleton transport and membrane targeting: cytoskeletal proteins	Poster	Sun AM				
28.	Cytoskeleton transport and membrane targeting: organelles and proteins	Poster	Sun AM				
29.	Gene structure and function I	Poster	Sun AM				
332.	Gene structure and function II	Poster		Mon PM			
497.	Gene structure and function III	Slide			Tue PM		
521.	Gene structure and function IV	Poster			Tue PM		
710.	Gene structure and function V	Poster				Wed PM	
519.	Membrane composition and cell-surface macromolecules I	Poster			Tue PM		
520.	Membrane composition and cell-surface macromolecules II	Poster			Tue PM		
8.	Neuroglia and myelin I	Slide	Sun AM				
134.	Neuroglia and myelin II	Poster	Sun PM				
135.	Neuroglia and myelin III	Poster	Sun PM				
239.	Neuroglia and myelin IV	Poster		Mon AM			
428.	Neuroglia and myelin V	Poster			Tue AM		
238.	Staining, tracing, and imaging techniques I	Poster		Mon AM			
427.	Staining, tracing, and imaging techniques II	Poster			Tue AM		
708.	Staining, tracing, and imaging techniques III	Poster				Wed PM	

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
709.	Staining, tracing, and imaging techniques IV	Poster				Wed PM	
	THEME C: EXCITABLE MEMBRANES AND SYNAPTIC TRANSMISSION						
508.	Calcium channel structure, function and expression I	Slide			Tue PM		
617.	Calcium channel structure, function and expression II	Poster				Wed AM	
618.	Calcium channel structure, function and expression III	Poster				Wed AM	
620.	Calcium channels: characterization and electrophysiology	Poster				Wed AM	
799.	Calcium channels: miscellaneous blockers	Poster					Thu AM
31.	Calcium channels: modulation by intracellular messengers	Poster	Sun AM				
141.	Calcium channels: modulation by non-peptide neurotransmitters	Poster	Sun PM				
140.	Calcium channels: modulation by peptide toxins	Poster	Sun PM				
619.	Calcium channels: modulation by peptides and hormones	Poster				Wed AM	
214.	Calcium channels: physiology, pharmacology and modulation I	Slide		Mon AM			
690.	Calcium channels: physiology, pharmacology and modulation II	Slide				Wed PM	
798.	Chloride and other channels	Poster					Thu AM
32.	Ion channels: cell function I	Poster	Sun AM				
33.	Ion channels: cell function II	Poster	Sun AM				
783.	Ion channels: cell function III	Slide					Thu AM
30.	Ligand-gated ion channels I	Poster	Sun AM				
498.	Ligand-gated ion channels II	Slide			Tue PM		
713.	Ligand-gated ion channels III	Poster				Wed PM	
111.	Long-term potentiation: pharmacology I	Slide	Sun PM				
522.	Long-term potentiation: pharmacology II	Poster			Tue PM		
523.	Long-term potentiation: pharmacology III	Poster			Tue PM		
245.	Long-term potentiation: physiology I	Poster		Mon AM			
246.	Long-term potentiation: physiology II	Poster		Mon AM			
433.	Long-term potentiation: physiology III	Poster			Tue AM		
434.	Long-term potentiation: physiology IV	Poster			Tue AM		
711.	Long-term potentiation: physiology V	Poster				Wed PM	
712.	Long-term potentiation: physiology VI	Poster				Wed PM	
788.	Long-term potentiation: physiology VII	Slide					Thu AM
680.	**Molecular Organization of the Postsynaptic Membrane**	**SYMP**				**Wed PM**	
777.	**Opioidergic Modulation of Long-term Potentiation in the Hippocampus: Insights Peptidergic Regulation of Synaptic Plasticity**	**SYMP**					**Thu AM**
333.	Pharmacology of synaptic transmission I	Poster		Mon PM			
615.	Pharmacology of synaptic transmission II	Poster				Wed AM	
616.	Pharmacology of synaptic transmission III	Poster				Wed AM	
240.	Postsynaptic mechanisms I	Poster		Mon AM			
241.	Postsynaptic mechanisms II	Poster		Mon AM			
242.	Postsynaptic mechanisms III	Poster		Mon AM			
243.	Postsynaptic mechanisms IV	Poster		Mon AM			
244.	Postsynaptic mechanisms V	Poster		Mon AM			
684.	Postsynaptic mechanisms VI	Slide				Wed PM	
209.	Potassium channel physiology, pharmacology and modulation I	Slide		Mon AM			
718.	Potassium channel physiology, pharmacology and modulation II	Poster				Wed PM	
719.	Potassium channel physiology, pharmacology and modulation III	Poster				Wed PM	

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
720.	Potassium channel physiology, pharmacology and modulation IV	Poster				Wed PM	
121.	Potassium channel structure, function and expression I	Slide	Sun PM				
412.	Potassium channel structure, function and expression II	Slide			Tue AM		
524.	Potassium channel structure, function and expression III	Poster			Tue PM		
525.	Potassium channel structure, function and expression IV	Poster			Tue PM		
18.	Presynaptic mechanisms I	Slide	Sun AM				
136.	Presynaptic mechanisms II	Poster	Sun PM				
137.	Presynaptic mechanisms III	Poster	Sun PM				
138.	Presynaptic mechanisms IV	Poster	Sun PM				
139.	Presynaptic mechanisms V	Poster	Sun PM				
430.	Presynaptic mechanisms VI	Poster			Tue AM		
431.	Presynaptic mechanisms VII	Poster			Tue AM		
432.	Presynaptic mechanisms VIII	Poster			Tue AM		
599.	Presynaptic mechanisms IX	Slide				Wed AM	
714.	Sodium channels I	Poster				Wed PM	
715.	Sodium channels II	Poster				Wed PM	
716.	Sodium channels III	Poster				Wed PM	
717.	Sodium channels IV	Poster				Wed PM	
	THEME D: NEUROTRANSMITTERS, MODULATORS, TRANSPORTERS, AND RECEPTORS						
34.	Acetylcholine	Poster	Sun AM				
801.	Acetylcholine receptor muscarinic: agonist/antagonist for receptors	Poster					Thu AM
800.	Acetylcholine receptor muscarinic: muscarinic receptors—molecular biology and electrophysiology	Poster					Thu AM
802.	Acetylcholine receptor muscarinic: receptors—expression	Poster					Thu AM
11.	Acetylcholine receptor: nicotinic I	Slide	Sun AM				
621.	Acetylcholine receptor: nicotinic II	Poster				Wed AM	
528.	Acetylcholine receptor: nicotinic expression	Poster			Tue PM		
527.	Acetylcholine receptor: nicotinic expression and treatment effects	Poster			Tue PM		
526.	Acetylcholine receptor: nicotinic—acetylcholine-effects of nicotine on specific brain regions	Poster			Tue PM		
334.	Acetylcholine receptor: nicotinic—acetylcholine-genetics: molecular	Poster		Mon PM			
247.	Acetylcholine receptor: nicotinic—acetylcholine-pharmacology and behavior	Poster		Mon AM			
143.	Acetylcholine receptor: nicotinic—acetylcholine-regulation, distribution, and physiology	Poster	Sun PM				
142.	Acetylcholine receptor: nicotinic—biophysics	Poster	Sun PM				
721.	Acetylcholine receptor: nicotinic—pharmacology	Poster				Wed PM	
36.	Acetylcholine receptor: nicotinic—structure/function	Poster	Sun AM				
35.	Acetylcholine: distribution	Poster	Sun AM				
839.	Acetylcholine: modulators	Poster					Thu AM
544.	Behavioral pharmacology I	Poster			Tue PM		
637.	Behavioral pharmacology II	Poster				Wed AM	
305.	Behavioral pharmacology: psychostimulants	Slide		Mon PM			
445.	Behavioral pharmacology: serotonin and dopamine	Poster			Tue AM		
632.	Catecholamine receptors: alpha adrenergic	Poster				Wed AM	
149.	Catecholamine receptors: antisense and knock outs	Poster	Sun PM				
633.	Catecholamine receptors: beta adrenergic	Poster				Wed AM	
441.	Catecholamine receptors: D_1 and D_5 pharmacology	Poster			Tue AM		
252.	Catecholamine receptors: D_2, D_3, D_4 pharmacology	Poster		Mon AM			

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
150.	Catecholamine receptors: distribution	Poster	Sun PM				
807.	Catecholamine receptors: genetics	Poster					Thu AM
340.	Catecholamine receptors: in vivo drug effects I	Poster		Mon PM			
341.	Catecholamine receptors: in vivo drug effects II	Poster		Mon PM			
253.	Catecholamine receptors: structure and function	Poster		Mon AM			
596.	Catecholamines	Slide				Wed AM	
446.	Catecholamines: biosynthetic enzymes	Poster			Tue AM		
448.	Catecholamines: electrophysiological studies	Poster			Tue AM		
43.	Catecholamines: gene structure and regulation	Poster	Sun AM				
447.	Catecholamines: microdialysis/voltammetric studies	Poster			Tue AM		
257.	Catecholamines: noradrenergic systems	Poster		Mon AM			
39.	Excitatory amino acid receptors I	Poster	Sun AM				
40.	Excitatory amino acid receptors II	Poster	Sun AM				
41.	Excitatory amino acid receptors III	Poster	Sun AM				
308.	Excitatory amino acid receptors IV	Slide		Mon PM			
335.	Excitatory amino acid receptors V	Poster		Mon PM			
336.	Excitatory amino acid receptors VI	Poster		Mon PM			
337.	Excitatory amino acid receptors VII	Poster		Mon PM			
338.	Excitatory amino acid receptors VIII	Poster		Mon PM			
437.	Excitatory amino acid receptors IX	Poster			Tue AM		
438.	Excitatory amino acid receptors X	Poster			Tue AM		
439.	Excitatory amino acid receptors XI	Poster			Tue AM		
623.	Excitatory amino acid receptors XII	Poster				Wed AM	
781.	Excitatory amino acid receptors XIII	Slide					Thu AM
722.	Excitatory amino acid receptors: receptor localization	Poster				Wed PM	
249.	Excitatory amino acids: anatomy and physiology I	Poster		Mon AM			
250.	Excitatory amino acids: anatomy and physiology II	Poster		Mon AM			
144.	Excitatory amino acids: anatomy and physiology—regional localization	Poster	Sun PM				
37.	Excitatory amino acids: excitotoxicity I	Poster	Sun AM				
38.	Excitatory amino acids: excitotoxicity II	Poster	Sun AM				
217.	Excitatory amino acids: excitotoxicity III	Slide		Mon AM			
248.	Excitatory amino acids: excitotoxicity IV	Poster		Mon AM			
409.	Excitatory amino acids: excitotoxicity V	Slide			Tue AM		
529.	Excitatory amino acids: excitotoxicity VI	Poster			Tue PM		
530.	Excitatory amino acids: excitotoxicity VII	Poster			Tue PM		
622.	Excitatory amino acids: excitotoxicity VIII	Poster				Wed AM	
803.	Excitatory amino acids: excitotoxicity IX	Poster					Thu AM
145.	Excitatory amino acids: pharmacology—drugs	Poster	Sun PM				
251.	Excitatory amino acids: pharmacology—mGluRs	Poster		Mon AM			
436.	Excitatory amino acids: pharmacology—NMDA	Poster			Tue AM		
499.	Excitatory amino acids: pharmacology—NMDA/AMPA receptors	Slide			Tue PM		
435.	Excitatory amino acids: pharmacology—secondary messenger	Poster			Tue AM		
146.	GABA receptors I	Poster	Sun PM				
339.	GABA receptors II	Poster		Mon PM			
531.	GABA receptors III	Poster			Tue PM		
624.	GABA receptors IV	Poster				Wed AM	
723.	GABA receptors V	Poster				Wed PM	
804.	GABA receptors VI	Poster					Thu AM
408.	GABA receptors: neuromodulators	Slide			Tue AM		
19.	GABA receptors: regulation and recombinant expression	Slide	Sun AM				
299.	**Gap Junctions in the Normal and Pathologic Nervous System**	SYMP		**Mon PM**			

Session Number	Session Title	Type	Day and Time Sun.	Mon.	Tue.	Wed.	Thu.
152.	Interactions between neurotransmitters I	Poster	Sun PM				
153.	Interactions between neurotransmitters II	Poster	Sun PM				
541.	Interactions between neurotransmitters III	Poster			Tue PM		
542.	Interactions between neurotransmitters IV	Poster			Tue PM		
584.	**Ligand-Gated Ion Channel Superfamily Feud**	SYMP				**Wed AM**	
3.	**Molecular Biological Studies of the Newly Cloned Opioid Receptors**	SYMP	**Sun AM**				
805.	Neuropeptide localization: CNS regulation	Poster					Thu AM
147.	Neuropeptide localization: endocrine peptides	Poster	Sun PM				
440.	Neuropeptide localization: sensory peptides	Poster			Tue AM		
306.	Neurotransmitter characterization and degradation	Slide		Mon PM			
725.	Neurotransmitter processing	Poster				Wed PM	
626.	Neurotransmitter release	Poster				Wed AM	
219.	Opioid receptors I	Slide		Mon AM			
784.	Opioid receptors II	Slide					Thu AM
533.	Opioid receptors: cell biology and physiology	Poster			Tue PM		
534.	Opioid receptors: development and regulation	Poster			Tue PM		
535.	Opioid receptors: localization	Poster			Tue PM		
629.	Opioid receptors: molecular biology	Poster				Wed AM	
630.	Opioid receptors: pharmacology	Poster				Wed AM	
631.	Opioid receptors: sigma receptors	Poster				Wed AM	
148.	Opioids: anatomy, physiology and behavior I	Poster	Sun PM				
536.	Opioids: anatomy, physiology and behavior II	Poster			Tue PM		
726.	Opioids: anatomy, physiology and behavior III	Poster				Wed PM	
151.	Other neurotransmitters: adenosine	Poster	Sun PM				
634.	Other neurotransmitters: GABA, glycine, purine	Poster				Wed AM	
729.	Other neurotransmitters: histamine	Poster				Wed PM	
810.	Other neurotransmitters: miscellaneous	Poster					Thu AM
254.	Other neurotransmitters: nitric oxide	Poster		Mon AM			
402.	Peptide receptor structure and function I	Slide			Tue AM		
532.	Peptide receptor structure and function II	Poster			Tue PM		
625.	Peptide receptor structure and function III	Poster				Wed AM	
724.	Peptide receptor structure and function IV	Poster				Wed PM	
42.	Peptides: physiological effects I	Poster	Sun AM				
627.	Peptides: physiological effects II	Poster				Wed AM	
628.	Peptides: physiological effects III	Poster				Wed AM	
806.	Peptides: physiological effects IV	Poster					Thu AM
210.	Peptides: physiology and anatomy	Slide		Mon AM			
44.	Receptor modulation, up- and down-regulation I	Poster	Sun AM				
449.	Receptor modulation, up- and down-regulation II	Poster			Tue AM		
635.	Regional localization of receptors and transmitters I	Poster				Wed AM	
636.	Regional localization of receptors and transmitters II	Poster				Wed AM	
313.	Second messengers I	Slide		Mon PM			
345.	Second messengers II	Poster		Mon PM			
346.	Second messengers III	Poster		Mon PM			
732.	Second messengers IV	Poster				Wed PM	
813.	Second messengers V	Poster					Thu AM
444.	Second messengers: calcium	Poster			Tue AM		
731.	Second messengers: G-proteins	Poster				Wed PM	
812.	Second messengers: kinases	Poster					Thu AM
809.	Serotonin	Poster					Thu AM
113.	Serotonin receptors I	Slide	Sun PM				
312.	Serotonin receptors II	Slide		Mon PM			
727.	Serotonin receptors: 5-HT_1	Poster				Wed PM	
443.	Serotonin receptors: 5-HT_2	Poster			Tue AM		
808.	Serotonin receptors: 5-HT_3	Poster					Thu AM

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
728.	Serotonin receptors: 5-HT_4, 5-HT_5, 5-HT_6, 5-HT_7	Poster				Wed PM	
539.	Serotonin receptors: autoreceptors	Poster			Tue PM		
538.	Serotonin receptors: behavior	Poster			Tue PM		
537.	Serotonin receptors: effectors	Poster			Tue PM		
442.	Serotonin receptors: structure and structure/function	Poster			Tue AM		
343.	Serotonin: development	Poster		Mon PM			
342.	Serotonin: regulation	Poster		Mon PM			
540.	Serotonin: uptake, function	Poster			Tue PM		
255.	Transmitters in invertebrates: neuropeptides	Poster		Mon AM			
256.	Transmitters in invertebrates: NO, amines, etc.	Poster		Mon AM			
154.	Uptake and transporters: catecholamines I	Poster	Sun PM				
543.	Uptake and transporters: catecholamines II	Poster			Tue PM		
730.	Uptake and transporters: glutamate	Poster				Wed PM	
811.	Uptake and transporters: miscellaneous	Poster					Thu AM
316.	Uptake and transporters: monoamines	Slide		Mon PM			
344.	Uptake and transporters: serotonin	Poster		Mon PM			
	THEME E: ENDOCRINE AND AUTONOMIC REGULATION						
14.	Cardiovascular regulation: central modulation	Slide	Sun AM				
549.	Cardiovascular regulation: determinants of sympathetic nerve response	Poster			Tue PM		
354.	Cardiovascular regulation: medullary reticular formation	Poster		Mon PM			
550.	Cardiovascular regulation: organization and pathology	Poster			Tue PM		
551.	Cardiovascular regulation: pharmacology	Poster			Tue PM		
403.	Cardiovascular regulation: spinalmedullary mechanisms	Slide			Tue AM		
258.	Cardiovascular regulation: supramedullary mechanisms	Poster		Mon AM			
259.	Cardiovascular regulation: vagal organization	Poster		Mon AM			
353.	Cardiovascular regulation: vagal pharmacology and physiology	Poster		Mon PM			
640.	Gastrointestinal regulation: gastroesophageal control	Poster				Wed AM	
641.	Gastrointestinal regulation: intestinal hepatic and pancreatic control	Poster				Wed AM	
218.	Hypothalamic-pituitary-adrenal regulation	Slide		Mon AM			
546.	Hypothalamic-pituitary-adrenal regulation: CRF	Poster			Tue PM		
347.	Hypothalamic-pituitary-adrenal regulation: glucocorticoids	Poster		Mon PM			
545.	Hypothalamic-pituitary-adrenal regulation: other I	Poster			Tue PM		
638.	Hypothalamic-pituitary-adrenal regulation: other II	Poster				Wed AM	
46.	Hypothalamic-pituitary-gonadal regulation I	Poster	Sun AM				
112.	Hypothalamic-pituitary-gonadal regulation II	Slide	Sun PM				
405.	Hypothalamic-pituitary-gonadal regulation III	Slide			Tue AM		
742.	Hypothalamic-pituitary-gonadal regulation IV	Poster				Wed PM	
743.	Hypothalamic-pituitary-gonadal regulation V	Poster				Wed PM	
744.	Hypothalamic-pituitary-gonadal regulation VI	Poster				Wed PM	
745.	Hypothalamic-pituitary-gonadal regulation VII	Poster				Wed PM	
45.	Neural-immune interactions: cytokines I	Poster	Sun AM				
352.	Neural-immune interactions: cytokines II	Poster		Mon PM			
548.	Neural-immune interactions: depression and stress	Poster			Tue PM		
452.	Neural-immune interactions: inflammation	Poster			Tue AM		
814.	Neural-immune interactions: neurotransmitters and neuromodulators	Poster					Thu AM
590.	Neural-immune interactions: other	Slide				Wed AM	
451.	Neural-immune interactions: pathology	Poster			Tue AM		
547.	Neuroendocrine regulation: hypothalamic peptides	Poster			Tue PM		
639.	Neuroendocrine regulation: other I	Poster				Wed AM	

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
733.	Neuroendocrine regulation: other II	Poster				Wed PM	
782.	Neuroendocrine regulation: other III	Slide					Thu AM
351.	Neuroendocrine regulation: thyroid and gonadal axes	Poster		Mon PM			
450.	Neuroendocrine regulation: vasopressin and oxytocin	Poster			Tue AM		
348.	Osmoregulation: magnocellular endocrine neurons	Poster		Mon PM			
349.	Osmoregulation: mechanisms	Poster		Mon PM			
736.	Respiratory regulation: amino acid transmitters	Poster				Wed PM	
737.	Respiratory regulation: brainstem and spinal cord	Poster				Wed PM	
738.	Respiratory regulation: central chemoreception	Poster				Wed PM	
740.	Respiratory regulation: developmental mechanisms	Poster				Wed PM	
741.	Respiratory regulation: reflex mechanisms and pathways	Poster				Wed PM	
739.	Respiratory regulation: signal transduction in the carotid body	Poster				Wed PM	
492.	**Sensory Circumventricular Organs (CVOs): Body-Brain Coupling and Mechanisms of Afferent Signaling**	SYMP			**Tue PM**		
350.	Thermoregulation and fever	Poster		Mon PM			
734.	Urogenital regulation: bladder and micturition	Poster				Wed PM	
735.	Urogenital regulation: sexual function	Poster				Wed PM	
	THEME F: SENSORY SYSTEMS						
166.	Auditory systems: central anatomy I	Poster	Sun PM				
271.	Auditory systems: central anatomy II	Poster		Mon AM			
361.	Auditory systems: central anatomy III	Poster		Mon PM			
56.	Auditory systems: central physiology I	Poster	Sun AM				
165.	Auditory systems: central physiology II	Poster	Sun PM				
269.	Auditory systems: central physiology III	Poster		Mon AM			
270.	Auditory systems: central physiology IV	Poster		Mon AM			
461.	Auditory systems: central physiology V	Poster			Tue AM		
462.	Auditory systems: central physiology VI	Poster			Tue AM		
164.	Auditory, vestibular, and lateral line: periphery	Poster	Sun PM				
4.	**Cellular and Molecular Mechanisms of Integration in Mammalian Retina**	SYMP	**Sun AM**				
686.	Chemical senses	Slide				Wed PM	
647.	Damaged retinas	Poster				Wed AM	
651.	Gustatory senses	Poster				Wed AM	
505.	Invertebrate sensory and motor	Slide			Tue PM		
167.	Invertebrate sensory systems I	Poster	Sun PM				
168.	Invertebrate sensory systems II	Poster	Sun PM				
463.	Olfactory senses: accessory olfactory system	Poster			Tue AM		
58.	Olfactory senses: invertebrates	Poster	Sun AM				
464.	Olfactory senses: olfactory bulb	Poster			Tue AM		
465.	Olfactory senses: olfactory cortex	Poster			Tue AM		
57.	Olfactory senses: olfactory receptor cells	Poster	Sun AM				
160.	Pain modulation: anatomy and physiology—behavior	Poster	Sun PM				
644.	Pain modulation: anatomy and physiology—brainstem	Poster				Wed AM	
261.	Pain modulation: anatomy and physiology—higher centers	Poster		Mon AM			
643.	Pain modulation: anatomy and physiology—human studies	Poster				Wed AM	
356.	Pain modulation: anatomy and physiology—neuropathic pain	Poster		Mon PM			
159.	Pain modulation: anatomy and physiology—periacqueductal gray	Poster	Sun PM				
262.	Pain modulation: anatomy and physiology—receptor and nerve	Poster		Mon AM			

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
158.	Pain modulation: anatomy and physiology—spinal cord I	Poster	Sun PM				
552.	Pain modulation: anatomy and physiology—spinal cord II	Poster			Tue PM		
459.	Pain modulation: pharmacology—allodynia	Poster			Tue AM		
263.	Pain modulation: pharmacology—amino acids, adenosine, cannabinoids	Poster		Mon AM			
553.	Pain modulation: pharmacology—anesthetics	Poster			Tue PM		
357.	Pain modulation: pharmacology—inflammation and prostaglandins	Poster		Mon PM			
554.	Pain modulation: pharmacology—neuropeptides	Poster			Tue PM		
645.	Pain modulation: pharmacology—NMDA and NO	Poster				Wed AM	
457.	Pain modulation: pharmacology—opioids I	Poster			Tue AM		
458.	Pain modulation: pharmacology—opioids II	Poster			Tue AM		
555.	Pain modulation: pharmacology—serotonin, histamine, catecholamines	Poster			Tue PM		
355.	Pain pathways: gene expression	Poster		Mon PM			
642.	Pain pathways: human studies	Poster				Wed AM	
260.	Pain pathways: peripheral nerves and spinal cord	Poster		Mon AM			
456.	Pain pathways: supraspinal centers	Poster			Tue AM		
694.	Pain: pathways and modulation	Slide				Wed PM	
104.	**Patterns of Activity Shaped by Local Circuitry in Mammalian Visual Cortex**	**SYMP**	**Sun PM**				
460.	Photoreceptors and RPE	Poster			Tue AM		
211.	Retina I	Slide		Mon AM			
413.	Retina II	Slide			Tue AM		
556.	Retina III	Poster			Tue PM		
646.	Retinal function	Poster				Wed AM	
161.	Retinal subcellular mechanisms I	Poster	Sun PM				
358.	Retinal subcellular mechanisms II	Poster		Mon PM			
155.	Sensory systems: spinal cord I	Poster	Sun PM				
156.	Sensory systems: spinal cord II	Poster	Sun PM				
157.	Sensory systems: spinal cord III	Poster	Sun PM				
455.	Somatic and visceral afferents: mechanoreceptors	Poster			Tue AM		
454.	Somatic and visceral afferents: nociceptors	Poster			Tue AM		
453.	Somatic and visceral afferents: visceral afferents	Poster			Tue AM		
50.	Somatosensory cortex and thalamocortical relationships I	Poster	Sun AM				
51.	Somatosensory cortex and thalamocortical relationships II	Poster	Sun AM				
52.	Somatosensory cortex and thalamocortical relationships III	Poster	Sun AM				
404.	Somatosensory cortex and thalamocortical relationships IV	Slide			Tue AM		
692.	Somatosensory cortex and thalamocortical relationships V	Slide				Wed PM	
53.	Somatosensory cortex and thalamocortical relationships: barrels I	Poster	Sun AM				
54.	Somatosensory cortex and thalamocortical relationships: barrels II	Poster	Sun AM				
47.	Subcortical somatosensory pathways I	Poster	Sun AM				
48.	Subcortical somatosensory pathways II	Poster	Sun AM				
49.	Subcortical somatosensory pathways III	Poster	Sun AM				
12.	Subcortical visual pathways I	Slide	Sun AM				
264.	Subcortical visual pathways II	Poster		Mon AM			
265.	Subcortical visual pathways III	Poster		Mon AM			
266.	Subcortical visual pathways IV	Poster		Mon AM			
693.	Visual cortex: extrastriate—attention	Slide				Wed PM	
120.	Visual cortex: extrastriate—dorsal stream I	Slide	Sun PM				
268.	Visual cortex: extrastriate—dorsal stream II	Poster		Mon AM			

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
359.	Visual cortex: extrastriate—functional organization I	Poster		Mon PM			
360.	Visual cortex: extrastriate—functional organization II	Poster		Mon PM			
504.	Visual cortex: extrastriate—functional organization III	Slide			Tue PM		
15.	Visual cortex: extrastriate—ventral stream I	Slide	Sun AM				
267.	Visual cortex: extrastriate—ventral stream II	Poster		Mon AM			
17.	Visual cortex: striate I	Slide	Sun AM				
162.	Visual cortex: striate II	Poster	Sun PM				
163.	Visual cortex: striate III	Poster	Sun PM				
311.	Visual cortex: striate IV	Slide		Mon PM			
592.	Visual cortex: striate V	Slide				Wed AM	
648.	Visual cortex: striate VI	Poster				Wed AM	
649.	Visual cortex: striate VII	Poster				Wed AM	
650.	Visual cortex: striate VIII	Poster				Wed AM	
689.	Visual cortex: striate IX	Slide				Wed PM	
55.	Visual psychophysics and behavior I	Poster	Sun AM				
212.	Visual psychophysics and behavior II	Slide		Mon AM			
	THEME G: MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION						
750.	Basal ganglia: anatomy	Poster				Wed PM	
817.	Basal ganglia: behavior	Poster					Thu AM
749.	Basal ganglia: drugs of abuse	Poster				Wed PM	
560.	Basal ganglia: movement disorders and experimental models	Poster			Tue PM		
170.	Basal ganglia: neuron activity during behavior	Poster	Sun PM				
653.	Basal ganglia: nigra and related systems	Poster				Wed AM	
362.	Basal ganglia: physiology	Poster		Mon PM			
272.	Basal ganglia: primate anatomy	Poster		Mon AM			
559.	Basal ganglia: striatal anatomy	Poster			Tue PM		
748.	Basal ganglia: striatal systems	Poster				Wed PM	
466.	Basal ganglia: thalamus	Poster			Tue AM		
652.	Basal ganglia: ventral striatal/ventral pallidal systems	Poster				Wed AM	
115.	Cerebellum	Slide	Sun PM				
467.	Cerebellum: anatomy and pharmacology	Poster			Tue AM		
363.	Cerebellum: behavior, development, models	Poster		Mon PM			
364.	Cerebellum: clinical studies	Poster		Mon PM			
818.	Cerebellum: genetic models	Poster					Thu AM
751.	Cerebellum: physiology	Poster				Wed PM	
696.	Circuitry and pattern generation	Slide				Wed PM	
64.	Circuitry and pattern generation: models and methods	Poster	Sun AM				
66.	Circuitry and pattern generation: modulation of CPG	Poster	Sun AM				
65.	Circuitry and pattern generation: simple systems	Poster	Sun AM				
277.	Circuitry and pattern generation: spinal cord	Poster		Mon AM			
819.	Control of posture and movement: human locomotion	Poster					Thu AM
172.	Control of posture and movement: injury and disease	Poster	Sun PM				
173.	Control of posture and movement: locomotion	Poster	Sun PM				
274.	Control of posture and movement: modelling	Poster		Mon AM			
114.	Control of posture and movement: motor control I	Slide	Sun PM				
276.	Control of posture and movement: motor control II	Poster		Mon AM			
562.	Control of posture and movement: motor units and reflexes	Poster			Tue PM		
275.	Control of posture and movement: posture I	Poster		Mon AM			
472.	Control of posture and movement: posture II	Poster			Tue AM		
755.	Control of posture and movement: prehension	Poster				Wed PM	
174.	Control of posture and movement: reaching I	Poster	Sun PM				

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
756.	Control of posture and movement: reaching II	Poster				Wed PM	
175.	Invertebrate motor functions	Poster	Sun PM				
169.	Motor cortex: anatomy	Poster	Sun PM				
815.	Motor cortex: behavioral physiology, models I	Poster					Thu AM
816.	Motor cortex: behavioral physiology, models II	Poster					Thu AM
746.	Motor cortex: functional organization and plasticity I	Poster				Wed PM	
747.	Motor cortex: functional organization and plasticity II	Poster				Wed PM	
557.	Motor cortex: human studies I	Poster			Tue PM		
558.	Motor cortex: human studies II	Poster			Tue PM		
215.	Motor systems: cortex	Slide		Mon AM			
563.	Muscle: biomechanics	Poster			Tue PM		
654.	Muscle: cellular and molecular physiology	Poster				Wed AM	
300.	**New Vistas in the Control of Arm Movement Trajectories**	SYMP		**Mon PM**			
753.	Oculomotor system: brainstem and pretectum	Poster				Wed PM	
367.	Oculomotor system: clinical studies	Poster		Mon PM			
470.	Oculomotor system: head movements and Listing's Law	Poster			Tue AM		
754.	Oculomotor system: near response, blink, and muscle	Poster				Wed PM	
502.	Oculomotor system: saccades	Slide			Tue PM		
366.	Oculomotor system: saccades—behavior and imaging	Poster		Mon PM			
469.	Oculomotor system: saccades—cortex	Poster			Tue AM		
468.	Oculomotor system: saccades—superior colliculus	Poster			Tue AM		
61.	Oculomotor system: smooth movements	Poster	Sun AM				
216.	Oculomotor system: smooth pursuit and vestibuloocular reflex	Slide		Mon AM			
561.	Reflex function: animal studies	Poster			Tue PM		
273.	Reflex function: human studies	Poster		Mon AM			
369.	Spinal cord and brainstem: anatomy	Poster		Mon PM			
63.	Spinal cord and brainstem: cellular neurophysiology	Poster	Sun AM				
471.	Spinal cord and brainstem: functional neurophysiology	Poster			Tue AM		
62.	Spinal cord and brainstem: pattern generation	Poster	Sun AM				
368.	Spinal cord and brainstem: pharmacology and transmitters	Poster		Mon PM			
171.	Spinal cord and brainstem: spinal reflexes	Poster	Sun PM				
365.	Vestibular system: nerve and nuclei	Poster		Mon PM			
752.	Vestibular system: vestibular nuclei	Poster				Wed PM	
59.	Vestibular system: vestibuloocular reflex—human studies	Poster	Sun AM				
60.	Vestibular system: vestibuloocular reflex—physiology and behavior	Poster	Sun AM				

THEME H: OTHER SYSTEMS OF THE CNS

371.	Association cortex and thalamocortical relations: anatomy	Poster		Mon PM			
372.	Association cortex and thalamocortical relations: physiology	Poster		Mon PM			
474.	Brain metabolism and blood flow: methods	Poster			Tue AM		
178.	Brain metabolism and blood flow: nitric oxide	Poster	Sun PM				
68.	Brain metabolism and blood flow: pharmacology	Poster	Sun AM				
656.	Brain metabolism and blood flow: physiology and biochemistry	Poster				Wed AM	
67.	Comparative neuroanatomy: forebrain I	Poster	Sun AM				
177.	Comparative neuroanatomy: forebrain II	Poster	Sun PM				
278.	Comparative neuroanatomy: non-forebrain	Poster		Mon AM			
655.	Limbic system and hypothalamus: amygdala and hypothalamus	Poster				Wed AM	
370.	Limbic system and hypothalamus: anatomy	Poster		Mon PM			

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
176.	Limbic system and hypothalamus: chemical anatomy	Poster	Sun PM				
473.	Limbic system and hypothalamus: function I	Poster			Tue AM		
564.	Limbic system and hypothalamus: function II	Poster			Tue PM		
THEME I: NEURAL BASIS OF BEHAVIOR							
82.	Aging: animal models	Poster	Sun AM				
192.	Aging: cell biology	Poster	Sun PM				
668.	Aging: primates including humans	Poster				Wed AM	
587.	Biological rhythms and sleep	Slide				Wed AM	
483.	Biological rhythms and sleep: aging	Poster			Tue AM		
76.	Biological rhythms and sleep: anatomy	Poster	Sun AM				
484.	Biological rhythms and sleep: disorders and clinical studies	Poster			Tue AM		
78.	Biological rhythms and sleep: melatonin and pineal	Poster	Sun AM				
658.	Biological rhythms and sleep: molecular and cellular biology	Poster				Wed AM	
77.	Biological rhythms and sleep: neurotransmitters and hormones I	Poster	Sun AM				
184.	Biological rhythms and sleep: neurotransmitters and hormones II	Poster	Sun PM				
185.	Biological rhythms and sleep: neurotransmitters and hormones III	Poster	Sun PM				
379.	Biological rhythms and sleep: physiology I	Poster		Mon PM			
380.	Biological rhythms and sleep: physiology II	Poster		Mon PM			
116.	Cognition I	Slide	Sun PM				
179.	Cognition II	Poster	Sun PM				
180.	Cognition III	Poster	Sun PM				
279.	Cognition IV	Poster		Mon AM			
373.	Cognition V	Poster		Mon PM			
374.	Cognition VI	Poster		Mon PM			
475.	Cognition VII	Poster			Tue AM		
476.	Cognition VIII	Poster			Tue AM		
588.	Cognition IX	Slide				Wed AM	
695.	Cognition X	Slide				Wed PM	
757.	Cognition XI	Poster				Wed PM	
779.	Cognition XII	Slide					Thu AM
820.	Cognition XIII	Poster					Thu AM
206.	Drugs of abuse: alcohol I	Slide		Mon AM			
485.	Drugs of abuse: alcohol II	Poster			Tue AM		
486.	Drugs of abuse: alcohol III	Poster			Tue AM		
665.	Drugs of abuse: alcohol IV	Poster				Wed AM	
666.	Drugs of abuse: alcohol V	Poster				Wed AM	
825.	Drugs of abuse: alcohol VI	Poster					Thu AM
824.	Drugs of abuse: alcohol and benzodiazepines	Poster					Thu AM
384.	Drugs of abuse: amphetamines and other stimulants I	Poster		Mon PM			
385.	Drugs of abuse: amphetamines and other stimulants II	Poster		Mon PM			
573.	Drugs of abuse: amphetamines and other stimulants III	Poster			Tue PM		
574.	Drugs of abuse: amphetamines and other stimulants IV	Poster			Tue PM		
826.	Drugs of abuse: amphetamines and other stimulants V	Poster					Thu AM
285.	Drugs of abuse: cocaine I	Poster		Mon AM			
286.	Drugs of abuse: cocaine II	Poster		Mon AM			
287.	Drugs of abuse: cocaine III	Poster		Mon AM			
288.	Drugs of abuse: cocaine IV	Poster		Mon AM			
765.	Drugs of abuse: cocaine V	Poster				Wed PM	
766.	Drugs of abuse: cocaine VI	Poster				Wed PM	

Session Number	Session Title	Type	Day and Time				
			Sun.	Mon.	Tue.	Wed.	Thu.
767.	Drugs of abuse: cocaine VII	Poster				Wed PM	
283.	Drugs of abuse: cocaine—development I	Poster		Mon AM			
284.	Drugs of abuse: cocaine—development II	Poster		Mon AM			
289.	Drugs of abuse: opioids and others I	Poster		Mon AM			
292.	Drugs of abuse: opioids and others II	Poster		Mon AM			
290.	Drugs of abuse: opioids and others—heroin and cannabinoids	Poster		Mon AM			
291.	Drugs of abuse: opioids and others—morphine	Poster		Mon AM			
572.	Hormonal control of reproductive behavior: behavioral measures	Poster			Tue PM		
282.	Hormonal control of reproductive behavior: functional anatomy	Poster		Mon AM			
190.	Hormonal control of reproductive behavior: parenting and sex differences	Poster	Sun PM				
80.	Hormonal control of reproductive behavior: receptors, chemistry, and anatomy	Poster	Sun AM				
188.	Ingestive behavior I	Poster	Sun PM				
280.	Ingestive behavior II	Poster		Mon AM			
383.	Ingestive behavior III	Poster		Mon PM			
410.	Ingestive behavior IV	Slide			Tue AM		
571.	Ingestive behavior V	Poster			Tue PM		
660.	Ingestive behavior VI	Poster				Wed AM	
10.	Ingestive behavior—peptides	Slide	Sun AM				
407.	Invertebrate learning and behavior I	Slide			Tue AM		
500.	Invertebrate learning and behavior II	Slide			Tue PM		
569.	Invertebrate learning and behavior III	Poster			Tue PM		
570.	Invertebrate learning and behavior IV	Poster			Tue PM		
659.	Invertebrate learning and behavior V	Poster				Wed AM	
69.	Learning and memory: pharmacology I	Poster	Sun AM				
70.	Learning and memory: pharmacology II	Poster	Sun AM				
71.	Learning and memory: pharmacology III	Poster	Sun AM				
377.	Learning and memory: pharmacology IV	Poster		Mon PM			
481.	Learning and memory: pharmacology V	Poster			Tue AM		
482.	Learning and memory: pharmacology VI	Poster			Tue AM		
375.	Learning and memory: physiology I	Poster		Mon PM			
376.	Learning and memory: physiology II	Poster		Mon PM			
758.	Learning and memory: physiology III	Poster				Wed PM	
759.	Learning and memory: physiology IV	Poster				Wed PM	
117.	Learning and memory: systems and functions I	Slide	Sun PM				
303.	Learning and memory: systems and functions II	Slide		Mon PM			
477.	Learning and memory: systems and functions III	Poster			Tue AM		
478.	Learning and memory: systems and functions IV	Poster			Tue AM		
479.	Learning and memory: systems and functions V	Poster			Tue AM		
480.	Learning and memory: systems and functions VI	Poster			Tue AM		
565.	Learning and memory: systems and functions VII	Poster			Tue PM		
566.	Learning and memory: systems and functions VIII	Poster			Tue PM		
567.	Learning and memory: systems and functions IX	Poster			Tue PM		
586.	Learning and memory: systems and functions X	Slide				Wed AM	
760.	Learning and memory: systems and functions XI	Poster				Wed PM	
761.	Learning and memory: systems and functions XII	Poster				Wed PM	
762.	Learning and memory: systems and functions XIII	Poster				Wed PM	
763.	Learning and memory: systems and functions XIV	Poster				Wed PM	
662.	Monoamines and behavior: dopamine I	Poster				Wed AM	
821.	Monoamines and behavior: dopamine II	Poster					Thu AM
81.	Monoamines and behavior: mental disorders, models, and treatments	Poster	Sun AM				

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
822.	Monoamines and behavior: norepinephrine	Poster					Thu AM
663.	Monoamines and behavior: serotonin	Poster				Wed AM	
183.	Motivation and emotion: animal models	Poster	Sun PM				
657.	Motivation and emotion: biochemistry and pharmacology	Poster				Wed AM	
378.	Motivation and emotion: primates including humans	Poster		Mon PM			
75.	Motivation and emotion: self-stimulation	Poster	Sun AM				
778.	**Neural Control of Breathing**	SYMP					**Thu AM**
20.	Neural plasticity	Slide	Sun AM				
73.	Neural plasticity: lesions and recovery	Poster	Sun AM				
181.	Neural plasticity: LTP	Poster	Sun PM				
74.	Neural plasticity: molecules and pharmacology	Poster	Sun AM				
182.	Neural plasticity: structural correlates	Poster	Sun PM				
72.	Neural plasticity: synaptic properties	Poster	Sun AM				
79.	Neuroethology: electroreception	Poster	Sun AM				
568.	Neuroethology: fish	Poster			Tue PM		
187.	Neuroethology: invertebrates	Poster	Sun PM				
186.	Neuroethology: other taxa	Poster	Sun PM				
381.	Neuroethology: songbirds I	Poster		Mon PM			
382.	Neuroethology: songbirds II	Poster		Mon PM			
304.	Neuropeptides and behavior I	Slide		Mon PM			
664.	Neuropeptides and behavior II	Poster				Wed AM	
823.	Neuropeptides and behavior III	Poster					Thu AM
309.	Psychotherapeutic drugs	Slide		Mon PM			
386.	Psychotherapeutic drugs: antidepressants	Poster		Mon PM			
191.	Psychotherapeutic drugs: antipsychotics I	Poster	Sun PM				
667.	Psychotherapeutic drugs: antipsychotics II	Poster				Wed AM	
827.	Psychotherapeutic drugs: other	Poster					Thu AM
281.	Stress: behavior	Poster		Mon AM			
207.	Stress: HPA axis	Slide		Mon AM			
764.	Stress: neurochemistry	Poster				Wed PM	
189.	Stress: neurotransmitter systems	Poster	Sun PM				
661.	Stress: preclinical and clinical studies	Poster				Wed AM	
583.	**What Is the Specific Role of the Cerebellum in Cognition?**	SYMP				**Wed AM**	

THEME J: DISORDERS OF THE NERVOUS SYSTEM

580.	Alzheimer's disease: ApoE	Poster			Tue PM		
400.	Alzheimer's disease: ApoE and genetics	Slide			Tue AM		
295.	Alzheimer's disease: chemical neuroanatomy	Poster		Mon AM			
773.	Alzheimer's disease: cholinergic neuropharmacology	Poster				Wed PM	
581.	Alzheimer's disease: genetics and clinical studies	Poster			Tue PM		
673.	Alzheimer's disease: mechanisms of degeneration I	Poster				Wed AM	
787.	Alzheimer's disease: mechanisms of degeneration II	Slide					Thu AM
108.	Alzheimer's disease: neurofibrillary degeneration	Slide	Sun PM				
674.	Alzheimer's disease: neuronal injury and death	Poster				Wed AM	
774.	Alzheimer's disease: neuropharmacology	Poster				Wed PM	
296.	Alzheimer's disease: tau and neurofibrillary degeneration	Poster		Mon AM			
507.	Beta-amyloid: aggregation	Slide			Tue PM		
109.	Beta-amyloid: animal models I	Slide	Sun PM				
579.	Beta-amyloid: animal models II	Poster			Tue PM		
9.	Beta-amyloid: ApoE I	Slide	Sun AM				
670.	Beta-amyloid: ApoE II	Poster				Wed AM	
671.	Beta-amyloid: cellular effects I	Poster				Wed AM	

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
672.	Beta-amyloid: cellular effects II	Poster				Wed AM	
85.	Beta-amyloid: gene expression	Poster	Sun AM				
578.	Beta-amyloid: localization	Poster			Tue PM		
194.	Beta-amyloid: neuromodulation	Poster	Sun PM				
577.	Beta-amyloid: neuropathology I	Poster			Tue PM		
589.	Beta-amyloid: neuropathology II	Slide				Wed AM	
401.	Beta-amyloid: neurotoxicity	Slide			Tue AM		
193.	Beta-amyloid: neurotoxicity and aggregation	Poster	Sun PM				
294.	Beta-amyloid: processing I	Poster		Mon AM			
310.	Beta-amyloid: processing II	Slide		Mon PM			
86.	Beta-amyloid: protein interactions	Poster	Sun AM				
208.	Beta-amyloid: secretion I	Slide		Mon AM			
390.	Beta-amyloid: secretion II	Poster		Mon PM			
195.	Degenerative disease: Alzheimer's—cognitive function	Poster	Sun PM				
685.	Degenerative disease: other	Slide				Wed PM	
199.	Degenerative disease: other—ALS and dementias	Poster	Sun PM				
196.	Degenerative disease: other—genetics and mechanisms	Poster	Sun PM				
198.	Degenerative disease: other—Huntington's	Poster	Sun PM				
197.	Degenerative disease: other—metabolic and inflammatory	Poster	Sun PM				
503.	Degenerative disease: Parkinson's	Slide			Tue PM		
488.	Degenerative disease: Parkinson's—clinical	Poster			Tue AM		
489.	Degenerative disease: Parkinson's—mechanisms	Poster			Tue AM		
491.	Degenerative disease: Parkinson's—MPTP models	Poster			Tue AM		
487.	Degenerative disease: Parkinson's—other models	Poster			Tue AM		
490.	Degenerative disease: Parkinson's—pharmacology	Poster			Tue AM		
775.	Degenerative disease: Parkinson's—transplantation, pallidotomy and imaging	Poster				Wed PM	
293.	Developmental disorders I	Poster		Mon AM			
669.	Developmental disorders II	Poster				Wed AM	
829.	Developmental disorders III	Poster					Thu AM
575.	Epilepsy: animal models I	Poster			Tue PM		
597.	Epilepsy: animal models II	Slide				Wed AM	
768.	Epilepsy: animal models III	Poster				Wed PM	
84.	Epilepsy: anticonvulsant drugs I	Poster	Sun AM				
831.	Epilepsy: anticonvulsant drugs II	Poster					Thu AM
314.	Epilepsy: basic mechanisms I	Slide		Mon PM			
388.	Epilepsy: basic mechanisms II	Poster		Mon PM			
389.	Epilepsy: basic mechanisms III	Poster		Mon PM			
576.	Epilepsy: basic mechanisms IV	Poster			Tue PM		
771.	Epilepsy: basic mechanisms V	Poster				Wed PM	
772.	Epilepsy: basic mechanisms VI	Poster				Wed PM	
770.	Epilepsy: genetic models	Poster				Wed PM	
830.	Epilepsy: kindling	Poster					Thu AM
769.	Epilepsy: primate studies	Poster				Wed PM	
105.	**From Fos to Proteolysis: Molecular Events in Brain Ischemia**	**SYMP**	**Sun PM**				
16.	Genetic models of human neuropsychiatric disorders I	Slide	Sun AM				
83.	Genetic models of human neuropsychiatric disorders II	Poster	Sun AM				
387.	Genetic models of human neuropsychiatric disorders III	Poster		Mon PM			
828.	Genetic models of human neuropsychiatric disorders IV	Poster					Thu AM
97.	Infectious diseases: HIV—diagnosis and treatment	Poster	Sun AM				
96.	Infectious diseases: HIV—pathogenesis	Poster	Sun AM				

Session Number	Session Title	Type	Day and Time: Sun.	Mon.	Tue.	Wed.	Thu.
834.	Infectious diseases: other	Poster					Thu AM
391.	Ischemia: apoptosis and gene expression	Poster		Mon PM			
87.	Ischemia: enzymes	Poster	Sun AM				
88.	Ischemia: glia and edema	Poster	Sun AM				
89.	Ischemia: glucose, pH and temperature	Poster	Sun AM				
392.	Ischemia: glutamate	Poster		Mon PM			
92.	Ischemia: imaging	Poster	Sun AM				
91.	Ischemia: inflammation and coagulation	Poster	Sun AM				
90.	Ischemia: ionic mechanisms	Poster	Sun AM				
213.	Ischemia: ischemic tolerance	Slide		Mon AM			
675.	Ischemia: ischemic tolerance and stress proteins	Poster				Wed AM	
93.	Ischemia: models	Poster	Sun AM				
501.	Ischemia: molecular mechanisms	Slide			Tue PM		
411.	Ischemia: neuroprotection and trophic factors	Slide			Tue AM		
393.	Ischemia: nitric oxide and other transmitters	Poster		Mon PM			
394.	Ischemia: oxidative injury	Poster		Mon PM			
94.	Ischemia: trophic factors, peptides and hormones	Poster	Sun AM				
110.	Mental illness I	Slide	Sun PM				
677.	Mental illness II	Poster				Wed AM	
678.	Mental illness—depression	Poster				Wed AM	
98.	Mental illness—schizophrenia I	Poster	Sun AM				
297.	Mental illness—schizophrenia II	Poster		Mon AM			
835.	Mental illness—schizophrenia III	Poster					Thu AM
838.	Neuro-oncology: treatment	Poster					Thu AM
837.	Neuro-oncology: tumor biology	Poster					Thu AM
204.	**The Neurobiology of Early Trauma: Implications for the Pathophysiology of Mood and Anxiety Disorders**	SYMP		**Mon AM**			
582.	Neuromuscular diseases	Poster			Tue PM		
396.	Neuromuscular diseases: motoneurons	Poster		Mon PM			
598.	Neurotoxicity I	Slide				Wed AM	
786.	Neurotoxicity II	Slide					Thu AM
776.	Neurotoxicity: heavy metals	Poster				Wed PM	
99.	Neurotoxins I	Poster	Sun AM				
298.	Neurotoxins II	Poster		Mon AM			
679.	Neurotoxins III	Poster				Wed AM	
836.	Neurotoxins IV	Poster					Thu AM
307.	Trauma	Slide		Mon PM			
200.	Trauma models and cell biology	Poster	Sun PM				
676.	Trauma: miscellaneous	Poster				Wed AM	
95.	Trauma: spinal cord	Poster	Sun AM				
832.	Trauma: treatment I	Poster					Thu AM
833.	Trauma: treatment II	Poster					Thu AM
395.	Trauma: white matter	Poster		Mon PM			
OTHER							
100.	History of neuroscience	Poster	AM, PM	AM, PM	AM, PM	AM, PM	AM
102.	Teaching of neuroscience: computer programs and internet	Poster	AM, PM	AM, PM	AM, PM	AM, PM	AM
101.	Teaching of neuroscience: curriculum development	Poster	AM, PM	AM, PM	AM, PM	AM, PM	AM
103.	Teaching of neuroscience: laboratory courses and exercises	Poster	AM, PM	AM, PM	AM, PM	AM, PM	AM

637.9

BEHAVIORAL EFFECTS AND PENTYLENETETRAZOLE-INDUCED SEIZURE PROTECTION OF DIZOCILPINE AND LORAZEPAM ALONE OR IN COMBINATION IN MALE CD1 MICE. G.A. Pritchard*, J. S. Pratt and D. J. Greenblatt. Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, Boston, MA 02111.

To evaluate the potential interaction between the NMDA receptors and the GABAA receptor family, dizocilpine (DIZ) and lorazepam (LRZ), alone or in combination, were evaluated for their effects on a number of behavioral parameters as well as in a pentylenetetrazole (PTZ) seizure protection assay in male CD-1 mice. Animals were injected with DIZ or vehicle and 15 min later with LRZ or vehicle. Distance traveled, rears and stereotypy were measured using an activity monitor 40 min following the first injection for 20 min (4 x 5 min). The tail vein of each mouse was then cannulated and PTZ was infused until the animal had two tonic-clonic seizures. Behavioral effects of DIZ were observed only for the highest dose (0.1 mg/kg) and was evidenced by a significant (80%) increase in distance traveled during each of four intervals monitored. Rears and stereotypy were unaffected by DIZ at any concentration. Also, DIZ had no protective effect against PTZ-induced seizures. Lorazepam, however, significantly reduced horizontal activity, rears and stereotypy at all but the lowest concentration (0.2 mg/kg) in a dose-dependent manner. Similarly, LRZ had a dose-dependent protective effect against PTZ-induced seizures with all but the lowest concentration (0.2 mg/kg) significantly increasing the volume of PTZ needed to induce convulsions. When combined at the lowest and highest levels tested, DIZ and LRZ had effects similar to that of LRZ alone; no synergism was observed Furthermore, the eleveated horizontal activity induced by DIZ (0.1 mg/kg) was completely attenuated by LRZ (2.0 mg/kg).

637.10

DOSE-RESPONSE OF CYCLOPHOSPHAMIDE-INDUCED EMESIS IN THE FERRET. R.L. Wynn, A. Carter, R. Meszler*, L. Lao, B.M. Berman and R.H. Wong. Department of Family Medicine, School of Medicine, and Departments of Anatomy and Pharmacology, Dental School, University of Maryland, Baltimore, MD 21201

Nausea and vomiting are severe side-effects often associated with cancer chemotherapy and may affect treatment decisions. Cyclophosphamide is a commonly used chemotherapy agent for breast cancer and induces emesis in the ferret. In order to examine the emetogenic effect of cyclophosphamide, ferrets (1.0-1.8 kg) were placed under general anesthesia (isoflurane 5%-O_2 mixture) and were administered logarithmic doses of i.v. cyclophosphamide. The mean number (±SE) of emetic episodes and retches were: 2.2±0.9 episodes and 2.8±1.9 retches at 56 mg/kg, 7.3±3.2 and 30.5±17.5 at 100 mg/kg, 23.3±4.0 and 85.3±20.4 at 177 mg/kg, and 23.5±7.5 and 62.5±38.5 at 237 mg/kg. In addition, various antiemetics were given i.v. immediately following cyclophosphamide injection. Ondansetron reduced emetic episodes by 0% and 43% (0.04 and 0.07 mg/kg), metoclopramide by 65% and 98% (4.08 and 7.07 mg/kg), and droperidol by 16% and 24% (0.45 and 0.25 mg/kg). These results indicate that cyclophosphamide induces emesis in a dose-dependent manner and may be useful in evaluating conventional and complementary therapies for the treatment of chemotherapy-induced emesis. Support provided by the U.S. Army Breast Cancer Research Program #DAMD17-94-J-4325.

637.11

EFFECTS OF I.C.V. AND I.C. BETA-FUNALTREXAMINE ON DISCRIMINATIVE STIMULUS EFFECTS OF MORPHINE. J.A. Moretti* and A.M. Young. Department of Psychology, Wayne State University, Detroit MI 48202.

The present experiments investigated effects of the selective irreversible μ opiate antagonist beta-funaltrexamine (β-FNA) on discriminative stimulus and rate-altering effects of morphine sulfate (MS), the prototypic μ opiate agonist. Male Sprague-Dawley rats were trained to discriminate among 3.2 mg/kg MS and saline under a FR15 schedule of food reinforcement. Generalization curves for MS (0.32-5.6 mg/kg) were established using a cumulative dosing procedure. Rats were then dosed with 20 μg β-FNA, by either i.c.v. or i.c. administration. Morphine was retested 24 h, 72 h, and 8 d after β-FNA treatment, and weekly thereafter, until recovery of the maximal stimulus effects of MS was observed. β-FNA i.c.v. produced a loss of maximal MS-like stimulus effects for up to 72 h, and also increased the dose of MS required to suppress rates by approximately six-fold. After 22 days the rate-altering potency of MS returned to control values, and MS regained the capacity to produce a maximal stimulus effect, although the dose required for MS-like responding was still greater than control values. The effects of β-FNA i.c. were significant, albeit less pronounced than those seen with i.c.v. administration, demonstrating that β-FNA is more potent when administered i.c.v. The present data suggest that β-FNA can suppress the maximal stimulus effects of MS by alkylating a sufficient proportion of the receptor pool mediating these responses. (Supported by DA03796 and K02 DA00132.)

637.12

STIMULUS ADDITIVITY IN A DRUG DISCRIMINATION TASK: CREATING A MORPHINE CUE FROM OTC COMPOUNDS.
D.V. Gauvin,* R.J. Briscoe, K.L. Carl and F.A. Holloway, Department of Psychiat. & Behav Sci, Univ. Oklahoma H.S.C., Okla. City, OK 73190

This laboratory recently has published reports demonstrating simple effect-additivity of stimulus elements of compound drug stimuli (*PB&B*, 32:1017-1023; *Psychopharm* 110:309-319; *J Psychopharm* 7:52-59). With respect to stimulus control by compound stimuli, Fantino & Logan have suggested that the specific stimulus element or dimension which controls the behavioral operant is largely beyond experimental control and may even be idiosyncratic (1979, The experimental analysis of behavior: a biological perspective San Francisco: W.H. Freeman). Using a two-choice drug discrimination task, rats were trained with 3.2 mg/kg morphine and saline as training stimuli. We used a single subject analysis strategy to examine which specific element(s) of the compound morphine cue controlled behavioral choice. Cross-generalization profiles were determined for a number of over-the-counter (OTC) medications which were hypothesized to engender subjective elements of a compound morphine cue including sedation, lethargy, and analgesia: dextromethorphan, doxylamine, diphenhydramine, pyrilamine, pyridoxine, thiamine, and loperamide. When tested in combination with saline, none of the OTC compounds produced a group mean percentage of 'drug-appropriate' responding > 50%. Based on an individual rats' cross-generalization profile, each subject was tested for 'stimulus-element-additivity' by administering and testing variations of binary and ternary combinations of these same OTC compounds. A test combination was formulated from the addition of single OTC drug stimulus elements for each rat which engendered >95% morphine-appropriate responding. These test combinations appeared to be idiosyncratic, dose-dependent, and to follow rules predicted by simple effect-additivity.

637.13

CUMULATIVE vs ACUTE DOSE-RESPONSE PROCEDURES PRODUCE DIFFERENTIAL BAC AND DD FUNCTIONS FOR ETOH
R.J. Briscoe,* D.V. Gauvin, K.A. Carl, & F.A. Holloway
Dept Psychiat & Behav Sci, O.U.H.S.C., Oklahoma City, OK 73190

Twelve male S-D rats were trained to discriminate between 1.25 g/kg ETOH and saline injections, in a multi-cycle operant discrimination task. Once trained, complete dose-effect curves (DECs) were generated, once per month, using two different incremented (cumulative) dose procedures (DEC1 & 3: 0.25, 0.75, 1.25, 1.75, 2.25 g/kg or DEC2 & 4: 0.0, 0.625, 1.25, 1.875, 2.25 g/kg). The same doses were tested singly in acute testing sessions over the five month period. Similar cumulative behavioral DECs were generated within each cumulative procedure (DEC#1 vs DEC#3, $F(1,6)$=.23: n.s.; DEC#2 vs DEC#4 $F(1,6)$=.13: n.s.) however, significant differences were produced between the two dosing incremented procedures (ED50s: DEC1 vs DEC3: 1.06 ± .25 vs 1.06 ± .23; DEC2 vs DEC4: .74 ± .15 vs .73 ± .17). Tests of the 1.25 g/kg ETOH training dose engendered 100% ETOH-appropriate responding under acute testing procedures but only ~70% ETOH-appropriate responding under cumulative testing procedures. When blood alcohol concentrations (BACs) were quantified by GC assays, cumulative testing procedures always produced significantly lower BACs than acute testing procedures ($t(6)$=8.12, p<.001). Group mean BACs produced by the 1.25 g/kg ETOH training dose were 70 ± 6 mg/dl and 126 ± 5 mg/dl when tested in cumulative and acute procedures, respectively. Interestingly, response rate functions did not differ between either cumulative procedures or between cumulative and acute procedures. These data may suggest that differential dosing procedures, interacting with ETOH-associated metabolic processes (absorption or distribution) may influence the behavioral choice and blood alcohol concentration DECs in rats.

637.14

TRAINING DOSE AS A DETERMINANT OF STIMULUS GENERALIZATION WITH A MIXTURE OF DRUGS. I. P. Stolerman*, E. A. Mariathasan and J-A W White. Section of Behavioural Pharmacology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, U.K.

The dose of a drug used for training can influence the characteristics of drug discrimination behaviour established with single drugs. To determine whether this also applies to discriminations based on mixtures of drugs, three groups of rats (n=9-10) were trained to discriminate mixtures of (+)-amphetamine (0.2-0.8 mg/kg) plus pentobarbitone (5-20 mg/kg) from saline in a standard, two-lever procedure with food reinforcement; the ratio between the training doses was constant at 1:25 for each group. The groups acquired the discriminations to an accuracy of 90-97% and, in each case, generalization to amphetamine or pentobarbitone alone was clear and dose-related, but less than complete in most experiments (68-86%). There was also partial generalization when either apomorphine (50%) or nicotine (63%) was administered alone, but only in rats trained on the smallest amount of the mixture; in rats trained with the larger doses of the mixture, neither apomorphine nor nicotine increased responding above vehicle levels. Doses of pentobarbitone that were half of those used in training produced little or no discriminative response when administered alone to the rats trained with the two smallest doses of the mixture; the same doses of pentobarbitone increased responses to amphetamine or apomorphine in a supra-additive manner. Strikingly, some doses of apomorphine and pentobarbitone that alone produced only vehicle-like responding (11%) produced almost complete generalization (80%) in rats trained with 0.4 mg/kg of amphetamine plus 10 mg/kg of pentobarbitone; this interaction was not seen in the rats trained with either smaller or larger doses of the mixture. The complex pattern of results suggests that doses of drugs used for training play an important role in the discrimination of abused drug mixtures, but no simple rules to predict the influence of training dose have been ascertained (supported by NIDA grant DA 05543).

637.15

HOTPLATE SENSITIVITY: INDIVIDUAL DIFFERENCES, HABITUATION, AND STIMULUS INTENSITY. D.F. Emerich*, M.A. Plone, and M.D. Lindner. CytoTherapeutics Inc., Two Richmond Square, Providence, RI 02906

The hotplate test, which typically involves placing a rodent in a chamber with a heated floor and recording the latency of the first sign of discomfort, is one of the most frequently used procedures in the study of pain and analgesics, yet the psychometric properties of this test have not been fully characterized. We conducted a survey of the literature which revealed that: (1) higher hotplate temperatures are more common than lower temperatures, and (2) studies do not match subjects into groups to ensure that groups are equivalent before treatment begins. It is likely that subjects are not matched into groups because it has been reported that "behavioral tolerance" occurs. We assessed morphine dose-response curves in rats that had been habituated or were naive to a 50°C or 55°C hotplate. The data reveal that: (1) consistent with numerous previous reports, the lower temperature hotplate was much more sensitive to the effects of analgesic agents such as morphine, (2) there are reliable differences between individuals, and (3) habituation to the hotplate testing procedures does not reduce the analgesic effects of morphine (there was no evidence of "behavioral tolerance" to the test procedures). These results support the use of lower stimulus intensities, and the practice of assessing pretreatment response latencies to the hotplate in order to match subjects into groups before treatments are initiated.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: OTHER II

638.1

THE PATTERN OF CELLULAR ACTIVATION SEEN IN RESPONSE TO ACUTE RESTRAINT SUGGESTS COMMONALITIES AMONG "NEUROGENIC" STRESS MODELS. V. Viau* and P.E. Sawchenko. The Salk Institute, La Jolla, CA 92037.

Acute restraint reliably activates the hypothalamo-pituitary-adrenal (HPA) axis, and is among the more commonly employed stress models. To provide an overview of the CNS systems that are activated in response to this challenge, we followed the time course of Fos protein expression induced in rat brain by a single 30 min restraint session. The widespread patterns of cellular activation we observed were most robust in animals sacrificed at 1-2 hr after stress, and returned to near control levels by 4 hr. Cell groups evincing Fos induction included the paraventricular nucleus of the hypothalamus (PVH), where responsive neurons were localized principally in the hypophysiotropic zone of parvocellular division, with secondary accumulations in autonomic-related subdivisions and in oxytocin-rich aspects of the magnocellular neurosecretory system. Beyond the hypothalamus, Fos-ir neurons could be grouped as belonging to three broadly defined systems. Induction was prominent in the limbic region of the telencephalon, including the infra- and prelimbic cortices, the lateral septal nucleus, bed nucleus of the stria terminalis, and the basolateral and medial amygdaloid nuclei. A second major focus comprised cell groups involved in the processing of somatosensory and/or nociceptive information; such structures included the dorsal column nuclei, the periaqueductal gray, cholinergic cell groups of the mesopontine tegmentum, and the midline and intralaminar nuclear complexes of the thalamus. Finally, brainstem catecholaminergic cell groups reliably displayed robust restraint-induced Fos expression. Prominent among these were the locus coeruleus, and cells in the medial part of the nucleus of the solitary tract, as well as aminergic regions of the rostral and caudal ventrolateral medulla. This general pattern of restraint-induced cellular activation is strikingly similar to that which has been described following footshock or immobilization, and contrasts starkly with profiles seen in response to systemic stresses such as hemorrhage or immune challenge. While the categorization of certain stress models as "neurogenic" appears to have some basis in induced patterns of immediate-early gene expression, it remains to be determined whether they activate the central limb of the HPA axis through common circuitries and mechanisms.

638.2

SEQUENCE OF STRESS-INDUCED ALTERATIONS IN INDICES OF CELLULAR ACTIVATION IN PARVOCELLULAR NEUROSECRETORY NEURONS: MARKERS VERSUS MECHANISMS. K.J. Kovács* and P.E. Sawchenko. The Salk Institute, La Jolla, CA and Inst. Exp. Med., Budapest, Hungary.

Acute ether stress results in increased ACTH and corticosterone secretion that peak at 5 and 30 min, respectively, after the challenge. Using cRNA probes to intronic sequences of genes encoding ACTH secretagogues in parvocellular neurosecretory neurons of the paraventricular nucleus (PVH), we have found these events to be accompanied by rapid and transient transcriptional activation of corticotropin-releasing factor (CRF) hnRNA expression (peak at 5-15 min), and a delayed up-regulation of arginine vasopressin (AVP) hnRNA (90-120 min). To gain insight into possible molecular mechanisms regulating stress-induced CRF and AVP expression *in vivo*, we have compared the time course of ether stress-induced activation of representatives of three transcription factor classes: immediate-early genes (*c-fos* and NGFI-B), a POU-domain factor (Brn-2), and the cAMP-response element binding protein (CREB), using antisera specific to its transcriptionally active, phosphorylated, form (P-CREB). Functional DNA binding sites for both Brn-2 and P-CREB have been identified on the CRF promoter. In response to ether stress, *c-fos* and NGFI-B mRNA induction in the PVH were maximal at 30-60 min, and that of Fos protein peaked at 90-120 min. Brn-2 mRNA was found to be constitutively expressed in parvocellular and magnocellular compartments of the PVH, and its expression did not change over the 4 hr observation period we employed. P-CREB was induced in parvocellular neurons with a time course which paralleled that of CRF hnRNA expression, reaching a maximum 5-15 min after exposure to ether vapor. *In vivo* inhibition of protein synthesis by cycloheximide prior to stress resulted in a marked reduction of the Fos protein response to ether in the parvocellular PVH, and attenuated the stress-induced rise in AVP hnRNA. Cycloheximide treatment did not, however, alter significantly P-CREB induction or stress-induced CRF hnRNA expression. The stress-induced transcriptional activation of the AVP and CRF genes in hypophysiotropic neurons appear to involve independent mechanisms, that respectively do and do not require *de novo* protein synthesis.

638.3

MECAMYLAMINE ADMINISTRATION INTO THE FOURTH VENTRICLE ANTAGONIZES NICOTINE-INDUCED c-FOS EXPRESSION IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS. B.M. Sharp, J.D. Valentine, K.M. McAllen, F.J. Wilson*, and S.G. Matta. Minneapolis Medical Research Foundation and Departments of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis, MN 55404 and Dept Neuroscience and Cell Biology UMDNJ-RW Johnson Medical School, Piscataway, NJ 08854

Previous research from this laboratory suggests that nicotine (NIC) elicits ACTH secretion indirectly, by activating brainstem neurons that project to the paraventricular nucleus of the hypothalamus (PVN), a region containing CRH. The present study further examined this hypothesis using a polysynaptic marker for neuronal activation, c-Fos. In experiment 1, rats received iv injections of vehicle or 0.045-0.18 NIC at a rate of 0.09 mg/kg/min and were sacrificed 1 hr later. Consistent with previous findings, immunohistochemical analysis for c-Fos showed that the PVN was dose-dependently activated by NIC, with the lower doses primarily activating the CRH rich parvocellular region and the higher doses activating the magnocellular region as well. In experiment 2, rats received vehicle or 4 ug of the nicotinic antagonist mecamylamine (MEC) into the fourth ventricle 15 min before iv administration of 0.09 mg/kg NIC. The results showed that MEC antagonized NIC-induced c-Fos expression throughout the PVN. These findings are consistent with prior results suggesting that NIC elicits ACTH release by activating brainstem neurons that in turn activate the PVN. (Supported by DA03977)

638.4

NICOTINE'S INDUCTION OF c-FOS IN THE NTS-A_2/C_2 IS ANTAGONIZED BY FOURTH VENTRICULAR MECAMYLAMINE. J.D. Valentine*, S.G. Matta, K.M. McAllen and B.M. Sharp. Minneapolis Medical Research Foundation and Departments of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis, MN 55404.

Previous research from this laboratory suggests that nicotine (NIC) elicits ACTH secretion by activating brainstem catecholamine neurons projecting to the paraventricular nucleus of the hypothalamus. The present study further examined this hypothesis. In experiment 1, rats received iv injections of vehicle, 0.045, 0.09, 0.135 or 0.18 mg/kg NIC at a rate of 0.09 mg/kg/min and were sacrificed 1 hr later. Immunohistochemical analysis showed that NIC dose-dependently induced c-Fos expression throughout the NTS-A_2/C_2. This expression was a linear function of dose from 0.045-0.135 mg/kg, with the 0.18 mg/kg dose causing a far greater induction. In experiment 2, rats received vehicle or 4 µg of the nicotinic antagonist mecamylamine (MEC) into the fourth ventricle 15 min before iv administration of 0.09 mg/kg NIC. The results showed that only a small fraction of the NTS-A_2/C_2 neurons expressing NIC-induced c-Fos also contained the catecholamine systhesis enzyme tyrosine hydroxylase. However, MEC antagonized NIC-induced c-Fos expression throughout the NTS-A_2/C_2. The present results are consistent with the hypothesis that NIC elicits ACTH secretion via activation of brainstem neurons, some of which are catecholaminergic. (Supported by DA03977)

638.5

INDUCTION OF THE IMMEDIATE-EARLY GENE c-*fos* IN THE AUDITORY AND LIMBIC SYSTEMS FOLLOWING LOUD NOISE STRESS. S. Campeau*, and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

The paraventricular nucleus of the hypothalamus is commonly thought to be the final common relay essential for stress-induced glucocorticoid release. However, the anatomical pathway(s) through which neurogenic stressors activate the paraventricular nucleus are not well delineated. In an attempt to determine more precisely how the paraventricular nucleus is activated by stress, a loud noise stimulus was used. Because the auditory system is already well characterized, it might provide a useful starting point to trace the pathway(s) ultimately innervating the paraventricular nucleus. Induction of c-fos mRNA was used to map the pattern of neural activation produced by loud noise.

Sprague-Dawley male rats (n = 4 per group) were used. Rats in one group served as naive controls. Rats in the other two groups were handled for 2 min and placed in the experimental cage for 10 min on each of 14 consecutive days. On Day 15, the animals were placed in the cage for 60 min, and for one group, a 110 dBA noise (20 - 20000 Hz) stimulus was turned on for the last 30 min. The rats were decapitated immediately upon removal from the cage, their trunk blood collected and processed for corticosterone radioimmunoassay. The brains were removed, frozen, and processed for c-*fos in situ* hybridization using a 680 nucleotide riboprobe.

Loud noise produced a reliable increase in plasma corticosterone concentration (20.3 ± 5.6 µg/dl) compared to handled/cage (0.6 ± 0.3 µg/dl) and naive controls (1.2 ± 0.4 µg/dl). Loud noise induced c-*fos* mRNA was observed in many forebrain and limbic areas as well as throughout the auditory system (from the cochlear nuclei to the auditory cortex). The pattern of activation in the forebrain and limbic systems parallels that obtained following swim and restraint stress (Cullinan et al., Neurosci., 64:477, 1995). Lesion studies are underway to determine which of the auditory relay nuclei provides the starting point of a multi-synaptic pathway ultimately innervating the paraventricular nucleus of the hypothalamus.

638.6

FOS IMMUNOREACTIVITY IN AMYGDALA AND HYPOTHALAMUS AFTER GRADED HEMORRHAGE IN THE RAT. M.P. Lilly, D.J. Putney and J.M. Simard*. University of Maryland at Baltimore, School of Medicine, Baltimore MD 21201.

We have previously reported potentiated responses of adrenocorticotropin (ACTH) to the second of two 10 ml/kg hemorrhages (hem) 24 h apart in the conscious rat. Limbic structures may modulate responses of the pituitary-adrenal (PA) system, and could play a role in potentiated PA responses. To study this, we measured fos-immunoreactivity (Fos-LIR) in brain regions after graded and/or repeated hem. Male Sprague-Dawley rats (350 gm) had chronic femoral arterial and venous catheters placed. Four days later conscious rats were bled 0, 5, 10 or 20 ml/kg over 3 min. Rats were killed 30, 60 or 90 min later and the brains were removed and frozen. Other rats had two 10 ml/kg hems 24 h apart and were killed 60 min p the second hem (10 ml/kg-H2). Fos immunostaining was done on 25µ frozen sections by the ABC method. Fos-LIR was evaluated qualitatively and semiquantitatively. Fos-LIR peaked 60 min after hem. No specific Fos-LIR was seen in brains after 0 or 5 ml/kg hem. After 10 ml/kg hem, we saw moderate Fos-LIR in the caudal central lateral (CeL) and the medial nuclei (MeA) of the amygdala, modest Fos-LIR in the supraoptic nucleus (SON) and no Fos-LIR in the paraventricular nucleus (PVN). After 20 ml/kg hem or 10 ml/kg-H2, we saw marked Fos-LIR throughout the central nucleus (CeA), SON and PVN and little Fos-LIR in MeA. These data show: 1) graded neuronal transcriptional activation after small hem, 2) repeated 10 ml/kg hem has a pattern of activation similar to 20 ml/kg hem, and 3) a lower threshold for hem activation of neurons in CeA & SON than in PVN. Thus, 10 ml/kg hem in the conscious rat activates cardiovascular neurons in amygdala, and a second 10 ml/kg hem produces more widespread neuronal activation. These data support the idea that amygdala neurons may play a role in potentiated PA responses to repeated hem. [Supported by NIH DK 02181]

638.7

TIME COURSE OF FOS STAINING FOLLOWING EXPOSURE TO RESTRAINT IN CHRONIC, INTERMITTENTLY COLD STRESSED ANIMALS. S. Bhatnagar* and M.F. Dallman, Dept. of Physiology, UCSF, San Francisco, CA 94143-0444.

Exposure of chronically stressed animals to a novel stressor results in similar or enhanced hypothalamic-pituitary-adrenal (HPA) responses to those in control animals despite the persistent negative feedback signal due to the chronic stress. It has been suggested that prior chronic stress produces a facilitatory trace that balances or overcomes the negative feedback effects of chronic stress, although the neuroanatomical sites subserving this facilitation are not yet known. We have shown that animals exposed to chronic, intermittent cold stress (4 h a day at 4C; CHR) exhibit enhanced HPA responses to a 30 min period of restraint compared to undisturbed controls (CTL). In the present study, we sought to determine the sites that may underlie this chronic stress-induced facilitation by immunohistochemistry for the fos protein. CHR and CTL animals were perfused under basal conditions, at 15 and 30 min during the 30 min period of restraint and at 30 and 60 min following termination of restraint. Preliminary data indicate that fos staining peaked in the paraventricular nucleus of the hypothalamus at 30 min post-restraint for both CTL and CHR animals, with no differences between groups in number of fos-stained cells at any time examined. Present data also indicate that staining in the arcuate nucleus is lower in CHR than in CTL animals at 30 min. Ongoing work is examining fos staining in other areas of the hypothalamus and brainstem with the goal of characterizing the neuroanatomical sites that may underlie chronic stress-induced facilitation of HPA responses to a novel stressor.

638.8

SEXUALLY DIMORPHIC MK801-INDUCED FOS-LIKE IMMUNOREACTIVITY (FLI) IN THE PVN OF THE RAT. N. Wintrip, D.M. Nance, and M. Wilkinson* Depts. of Obst. and Gynaecol., Physiol. and Biophys., and Anatomy and Neurobiology, Dalhousie U., Halifax, N.S., and Dept. of Pathology, U. of Manitoba, Winnipeg, Canada.

The non-competitive NMDA receptor antagonist MK801, is reported to activate the c-*fos* gene in various regions of the rat brain (Lee et al; Mol. Brain Res., 24:192). Also, the behavioural effects of NMDA appear to be sexually dimorphic (Hönack and Löscher; Brain Res., 620:167). We have examined induction of FLI in the PVN of male and female Sprague-Dawley rats 2h following s.c. injection of MK801 (0.1 or 1.0 mg/Kg b.w.; Fos antibody: Cambridge Res. Biochem.) using computer assisted image analysis. This approach has enabled us to identify subtle differences in the effects of MK801 which are difficult to detect by sequential examination of tissue sections.

Dose-dependent behavioural anomalies were seen in all MK801-injected rats with females exhibiting more pronounced disturbances at both high (1 mg/Kg) and low (0.1 mg/Kg) doses. Elevated levels of FLI were seen in the PVN of all MK801-treated rats compared to saline-injected controls. In general, high-dose (HD) MK801 induced higher levels of FLI in the PVN of adult male rats compared to like-treated cycling or lactating females (m.>fem.>lac.). Levels of FLI in the PVN of HD MK801-treated male and female 14 day old rats were similar and resembled those seen in adult males. In contrast, low dose (LD) MK801 induced more FLI in the PVN of cycling females compared to adult males. Gonadectomy did not affect the FLI response to LD MK801 in females but did increase levels in males. In adult female rats androgenized by testosterone (T) on postnatal day 2, levels of both HD and LD MK801-induced FLI in the PVN resembled those seen in like-treated adult males. These results suggest that the observed c-fos response to MK801 is T-dependent. Supported by Can. MRC.

638.9

EFFECT OF DURATION OF ADRENALECTOMY AND LIPOPOLY-SACCHARIDE ON C-FOS EXPRESSION IN THE PARAVENTRICULAR AND SUPRAOPTIC NUCLEI. A.A. Gerall*, S.B. Martin-Schild, and R.L. Chan. Dept. of Psychology, Tulane University, New Orleans, LA 70118.

Adrenalectomy (ADX) has been shown to enhance neuronal activity in the paraventricular nucleus of the hypothalamus (PVN) as measured by expression of c-fos-like protein (CFI) and CRH mRNA. Explored in the present study, were the influences of ADX duration and a pathogenic challenge on PVN and supraoptic nucleus (SON) expression of CFI.

Twenty-five intact or ADX Sprague-Dawley male rats were injected with either phosphate buffered saline (PBS) or 0.13 mg/kg bw lipopolysaccharide (LPS) 2 hr before perfusion. Experimental variables were: ADX duration, short term (<14 days) or long term (>21 days); and pre-perfusion challenge, control (PBS) or pathogen (LPS). Immunocytochemistry was performed using c-fos antiserum (Cambridge Biochem. Inc.) and solutions obtained from Vectastain Elite Sheep Kit. Immunoreactive products were counted using an computer-image analyzer.

Results confirm short term ADX non-challenged animals have significantly more CFI-ir in PVN neurons than intact animals. However, extending the duration of ADX before perfusion significantly diminished PVN CFI-ir. ADX resulted in more than 2x the CFI-ir in both the PVN and SON than in intact animals exposed to LPS. The main factors, ADX and LPS appear to be additive. LPS elicited a greater effect on neuronal activity in the PVN in long term ADX (14x) than short term ADX (3x). The difference was primarily due to increased baseline levels of CFI-ir within the parvocellular PVN of short term ADX PBS animals, which was 7x greater than that of long term ADX PBS animals. The duration of ADX had no influence over CFI-ir in the SON. Adjustment over time to ADX might result from a change in c-fos expression in parvocellular neurons but more likely is due to modifications in glucocorticoid receptor dynamics in CRH neurons or those regulating CRH neurons.

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638.10

CHRONIC CENTRAL ADMINISTRATION OF NEUROTENSIN: EFFECTS ON HYPOTHALAMIC-PITUITARY-ADRENAL ACTIVITY AND BEHAVIOR. Wayne B. Rowe[1]*, U.K. Hanisch[2], Shakti Sharma[1], Michael J. Meaney[1] and Rémi Quirion[1] [1]*Dept. of Psychiatry and Neurology/Neurosurgery, Douglas Hospital Research Center, McGill University, Montreal, Canada, H4H 1R3,* [2]*Max-Delbrück-Centrum, Robert-Rössle-Strasse 10, 13125 Berlin-Buch, Germany.*

Neuroendocrine studies suggest an important neuromodulatory role of neurotensin (NT) on hypothalamic-pituitary-adrencortical (HPA) function. NT produces significant increases in plasma ACTH and corticosterone (B) levels as early as 15 min following an acute central injection (1 pmol), and levels remain elevated for up to 4 hrs (Rowe et al., *J. Neuroendo.*, 1995, 7:109-117). We examined the HPA and behavioral effects of continous central NT infusion (1 pmol/hour for 14 days; via Alzet minipumps) into the lateral ventricle of male, 3 month old Long-Evans rats. Significant increases ($p < 0.05$) in basal HPA activity occurred during the dark phase of the diurnal cycle in the NT-treated animals compared to saline controls. At the end of the 14 day NT-infusion period, mineralocorticoid (MR) and glucocorticoid (GR) receptor levels were assessed in several brain areas. No differences in MR binding capacity were noted in hypothalamus, pituitary or hippocampus. A significant reduction ($p < 0.05$) in GR binding occurred in the hippocampus of chronic, NT-treated animals. NT- and saline-treated rats exhibited comparable ACTH and B levels during a 20-min immobilization stress; however, following termination of the stressor, plasma ACTH and B levels were significantly ($p < 0.05$) higher in the NT-treated group. This increase in HPA activity following stress may be associated with an impairment in HPA negative-feedback inhibition in the NT-treated animals. Taken together, these findings suggest that chronic NT treatment functions as a chronic stressor, elevating basal HPA activity, decreasing GR binding as well as altering negative-feedback and HPA responses to acute stressors. A significant decrease ($p < 0.05$) in mean locomotor activity was also noted in the chronically-treated NT animals following exposure to a novel environment. This decrease was restricted only to the first day of testing. As one of the behavioral charactistics of HPA hyperactivity is an increase in reactivity towards novelty (Stenzel-Poore et al., 1994, *J. Neurosci.*, 1994, 14: 2579-2584) it is possible that a NT-induced increase in HPA activity may in part be related to the behavioral response to novelty seen in these animals. Supported by MRC and FCAR.

638.11

DEVELOPMENTAL SHIFT IN ADRENERGIC RECEPTOR TYPE MEDIATING CATECHOLAMINE EFFECTS ON STRESS-INDUCED ACTH SECRETION IN THE RAT. C.-D. Walker*, D. Lavallée. McGill University, Douglas Hospital Research Center, Montréal, Canada.

During the first 2 weeks of life, rats exhibit low basal and stress-induced corticosterone (B) secretion and blunted adrenal sensitivity to ACTH. Maternal separation for 24h (SEP) increases basal adrenocortical activity, potentiates stress responses and most likely results in sympathetic activation. However, how increased catecholamine secretion following SEP could participate in the enhanced ACTH secretion is unknown. Before testing the effects of adrenergic blockade on ACTH secretion following SEP, we first used a 3min exposure to ether as an acute stressor known to stimulate catecholamine release. The effects of propranolol (3mg/kg,ip,-60min), prazosin (0.4, 2 mg/kg,ip,-60min) or idazoxan (1, 5 mg/kg,ip,-30min) pretreatment on basal and ether-induced ACTH and B secretion were determined in 9-10 days-old (d) and in 22-23d intact neonates. Saline pretreatment was used as a control. Blood samples were collected at 0, 5, and 30min after the onset of ether stress. Both prazosin and idazoxan increased basal and stimulated ACTH and B secretion at the highest doses in 10d neonates compared to saline-treated controls. Propranolol had no effect on ACTH or B release. In contrast, in 22d neonates, propranolol and prazosin decreased stress-induced ACTH secretion in an adult-like fashion while idazoxan tended to increase basal ACTH secretion. These results suggest that the control of neonatal ACTH secretion by alpha1-adrenergic receptors shifts from being inhibitory to mainly stimulatory with age. Alternatively, age-related changes in the distribution of hypothalamic adrenergic receptor types or efficacy of coupling to second messengers might dictate different effects on ACTH secretion in early neonatal life. We are now determining whether changes in central catecholamine release and/or alpha1-adrenergic receptors would affect the control of ACTH release following SEP in neonates. (Funded by NSERC and FCAR of Canada).

638.12

DISSOCIATION BETWEEN HORMONAL AND BEHAVIORAL STRESS RESPONSES TO THE FORCED SWIM TEST IN LACTATING RATS. D. Lavallée, G. Trottier, J. Rochford, P. Boksa*, C.-D. Walker. McGill University, Douglas Hospital Research Center, Montréal, Canada.

Lactation in the rat is associated with elevated corticosterone (B) release and blunted adrenocortical responses to stress. Because B secretion in the forced swim test (FST) at the time of the first swim (15min) is thought to facilitate immobility retention in a second session (5 min) 24h later, we determined whether lactating females would exhibit changes in immobility related to the magnitude of their basal and stress-induced ACTH and B secretion. Females in early (EL, d8-10) or late (LL, d17-19) lactation were compared to virgins or male rats for their acquisition and retention of immobility as well as for their ACTH and B responses to the FST. Intact lactating and virgin females were compared to their adrenalectomized (ADX, 5 days) or ovariectomized (OVX, 10 days) counterparts. Although ACTH and B responses to stress were similar in males and virgin females, immobility scores were greater in males than in virgins on both days of testing. Immobility retention occured in young males, but was not observed in any of the female groups, lactating or not. In contrast, basal B secretion was elevated in lactating females (EL>LL>V), the magnitude of the ACTH response was blunted in intact and ADX lactating females compared to virgins (V>EL>LL), but no differences in immobility scores were observed as a function of lactation. In OVX females (V or EL) immobility was decreased and basal ACTH and B was slightly reduced compared to intact rats. We conclude that: 1) dissociation between endocrine and behavioral responses occur as a function of sex and lactational status, questioning the facilitatory effect of B on immobility retention and 2) blunted stress responses in lactating females are not caused by increased basal B and thus enhanced inhibitory feedback on the adrenocortical system since they are still observed in ADX females. (Funded by MRC Canada).

638.13

WITHDRAWN

638.14

DIFFERENTIAL PROCESSING OF THE NEURO-ENDOCRINE PROTEIN "VGF" IN MAMMALIAN HYPOPHYSIS. G-L. Ferri*, R.M. Gaudio, I.F. Castello, A. Rinaldi#, A. Levi#, P. Berger°, R. Possenti# Univ. Dept. Cytomorphology, Cagliari & Oasi IRCCS, Troina, #CNR Inst. Neurobiology, Rome, Italy; °Inst. Biomed. Aging, Acad. Sciences, Innsbruck, A.

The neuro-endocrine protein "VGF" contains multiple potential cleavage sites and a cleavage-amidation site. Antisera to five VGF domains, plus the putative amidated sequence (*NH_2) were used in immunocytochemistry and Western blot. In Guinea-pig, bovine, pig and human (n=8-15/species) all sera stained anterior pituitary endocrine cells. As in rat, immuno-reactivity for the VGF domains was mainly found in lactotropes and/or gonadotropes, though incomplete overlap and inter-species differences were seen. Large, intermediate and small molecular weight forms were found in Western blot with most sera, while *NH_2-immunoreactivity was restricted to intermediate-small forms. In rat, no reactivity was found for the *NH_2-serum, in either immunocytochemistry or Western blot.

Thus, C-terminally amidated VGF fragments would be produced in a number of mammals. As for many regulatory peptides, C-terminal amidation may suggest biological significance of such *VGF* gene products.

638.15

HIGHLY SENSITIVE BRAINSTEM SITES FOR GLUCOPRIVIC STIMULATION OF THE ADRENAL MEDULLA. S. Ritter* and T.T. Dinh. Dept of VCAPP, Washington State University, Pullman, WA 99164-6520 USA.

Glucoreceptors controlling adrenal medullary secretion are known to be centrally located since administration of glucoprivic agents directly into the brain elicits a sympathoadrenal response. In addition, systemic glucoprivation elicits sympathoadrenal response in decerebrate rats, indicating that glucoreceptors are located in the hindbrain. The present study mapped the hindbrain between the facial nerve and obex of rats (n=70) for glucoreceptive sites. The antimetabolic glucose analogue, 5-thioglucose (5TG, 12-24 ug in 100-200 nl) and 0.9% saline were injected through small diameter cannulas. Blood was collected at 8 intervals during a 195 min period beginning 15 before the injection. At positive sites, blood glucose began to rise with short latency and peaked at about 60 min post injection. Positive sites were found to be closely associated with catecholamine (CA) cell groups, especially A1/C1 and C2, where 24 ug of 5TG raised glucose levels from 92 to 240 mg/dl. Surrounding sites, including ventricular sites, were less positive or negative. These hindbrain CA neurons may be involved in the adrenal medullary hyperglycemic response, since they express Fos-like immunoreactivity in response to glucoprivation and those of A1/C1 directly innervate adrenal medullary preganglionic neurons. However, additional experiments will be required to determine whether A1/C1 and C2 neurons are themselves glucoreceptive or whether other neurons in close proximity to these CA neurons are the putative glucoreceptor cells.

638.16

METYRAPONE'S EFFECTS ON CONDITIONED FEAR'S ENHANCEMENT OF MORPHINE ANALGESIA IN FEMALE SPRAGUE-DAWLEY RATS. H.S. Stock*, B.J. Caldarone, G.C. Abrahamsen, D.L. Mongeluzi, & R.A. Rosellini. Dept. of Psychology, University at Albany: SUNY, Albany, NY 12222.

A number of studies have reported that both the immediate and proactive effects of exposure to a shock stressor are less pronounced in female than in male rats. A separate area of research has demonstrated that female rats are less reactive to the analgesic effects of morphine than males. Experiments from our laboratory, as well as others, have found that exposure to a context associated with shock (i.e., conditioned fear context), at the time of morphine administration, enhances the analgesic effects of morphine. We have recently demonstrated that female rats show less pronounced conditioned fear-induced enhancement of morphine analgesia than male rats. The purpose of the present experiment was to begin to elucidate some of the hormonal mechanisms that may mediate this previously observed sex difference. In the present experiment female rats were injected with 75mg/kg of metyrapone, a corticosterone synthesis inhibitor. This dose has been demonstrated to suppress a female's stress-induced corticosterone levels to that of males. Three hours after injection the females were tested for enhancement of morphine analgesia during re-exposure to the conditioned fear context. The results demonstrated that females that were exposed to the conditioned fear context and pre-treated with metyrapone had higher morphine-induced analgesia than the control groups. The finding from this experiment suggests that corticosterone may play a role in mediating a female's response to conditioned fear-induced enhancement of morphine analgesia.

639.1

DEPRENYL INHIBITS THE DEVELOPMENT OF MAMMARY TUMORS IN RATS. S. ThyagaRajan*, S. Y. Felten and D. L. Felten, Dept. of Neurobiol. & Anat., Univ. of Rochester, Rochester, NY 14642.

Deprenyl, an irreversible monoamine oxidase-B (MAO-B) inhibitor, has been reported to decrease the incidence of spontaneous mammary tumors in old female rats by suppressing the metabolism of dopamine in the hypothalamus. Further, numerous studies have shown that deprenyl has a neuroprotective function independent of its MAO-B inhibitory activity. The purpose of this study was to investigate whether deprenyl can exert a neuroprotective effect in rats with dimethylbenzanthracene (DMBA)-induced mammary tumors. Female Sprague-Dawley rats (28-29 days old) were injected i.p. with 0.25 mg/kg or 2.5 mg/kg of deprenyl (Dep) or the vehicle (saline) daily for 4 weeks before the administration of DMBA. Following the administration of DMBA, the rats were treated with saline or deprenyl daily for 26 weeks . Below are the results of tumor number and body weight measurement at the end of the treatment period.

	Number of tumors/rat	Incidence of tumors	Body weight (gms)
Saline/Saline	2.8±0.3	87%	425±12
Dep 0.25/Saline	2.4±0.2	53%	409±14
Dep 2.5/Saline	2.4±0.3	53%	396±11
Saline/Dep 0.25	2.3±0.5	67%	431±15
Dep 0.25/Dep 2.5	1.7±0.2	60%	434±17
Saline/Dep 2.5	1.5±0.3	40%	396±10
Dep 2.5/Dep 2.5	1.4±0.2	33%	399±7

These results demonstrate that treatment with 0.25 mg and 2.5 mg of deprenyl pre- and post-administration of DMBA, and with 2.5 mg of deprenyl following the administration of DMBA causes inhibition of mammary tumor development in rats possibly by maintaining the activity of the tuberoinfundibular dopaminergic system in the hypothalamus.

639.2

TUMORS SECRETING PLACENTAL LACTOGEN-I (PL-I) OR HYPOTHALAMIC IMPLANTS OF LACTOGENIC HORMONES INHIBIT SUCKLING-INDUCED PROLACTIN BUT NOT THE ANTE-PARTUM PROLACTIN SURGE. R.J. Flietstra and J.L. Voogt*. Physiology Dept., Univ. of Kansas Med. Ctr., Kansas City, KS 66103.

Prolactin (PRL) secretion is inhibited by dopamine (DA) released from tuberoinfundibular dopaminergic (TIDA) neurons of the hypothalamus. High PRL levels stimulate TIDA neurons, inhibiting further PRL release. Other lactogenic hormones, specifically placental lactogens (PLs), can participate in this negative feedback loop. High circulating levels of PLs at midpregnancy terminate the twice daily PRL surges in female rats. In the dark period preceding parturition, however, an ante-partum PRL surge occurs despite continuously high levels of PL. Intrahypothalamic pituitary grafts also had no effect on this surge (Br. Res. Bul. 36:413, 1995). The aim of this study was to examine if the lactogenic hormone negative feedback loop is operational during the ante-partum surge. Sprague-Dawley rats were mated and received media or $1x10^6$ Rcho-1 (PL-I secreting) or HRP-1 cells (nonsecretors of lactogens) under the kidney capsule on the 10th day of pregnancy. Other pregnant rats received a hypothalamic implant of 25μg of albumin, ovine PRL or rat PL-I on day 19 or 20 of pregnancy. Serial blood samples were taken via carotid cannula from all rats. Two to 3 days following parturition, pups were removed for 4-6 hours, returned, and trunk blood samples taken after 1 hr suckling. Rcho-bearing rats (peak PRL level, 131 ng/ml) did not have a significantly reduced ante-partum PRL surge as compared to HRP-bearing (peak PRL level, 107 ng/ml). Yet suckling-induced PRL release was reduced by 86% in the Rcho-bearing dams as compared to the control dams ($p<.05$). Similarly, ante-partum PRL release was not reduced in rats given hypothalamic PRL (peak PRL level, 82 ng/ml) or PL-I (peak PRL level, 125 ng/ml) as compared to albumin-implanted dams (peak PRL level, 92ng/ml). However, suckling-induced PRL release was reduced more than 89% in both groups as compared to control rats ($p<.05$). This suggests that the lactogenic hormone negative feedback loop which is present during most of pregnancy and early lactation, is not operational just prior to parturition. Supported by NIH Grant HD 24190.

639.3

CHEMOTHERAPEUTIC EFFICACY: INFLUENCE OF PSYCHOSOCIAL FACTORS ON THE RESPONSE OF A MOUSE MAMMARY CARCINOMA (SC115). **L.R. Kerr, M.S. Grimm, W.A. Silva, T. P. O'Connor*, J.T. Emerman, J. Weinberg. Dept. of Anatomy, University of British Columbia, Vancouver, B.C., V6T 1Z3, Canada.**

In the present study, we demonstrated that psychosocial factors significantly altered the response of the transplantable androgen responsive Shionogi mouse mammary carcinoma (SC115) to adriamycin (AD) and cyclophosphamide (CY). Male DD/S mice were either reared individually then group housed (IG), or reared group housed and then singly housed (GI) following tumor cell or vehicle injection. Chemotherapy or drug vehicle injections (3 times at 7 day intervals) were started when tumors reached 1 g. To monitor the toxic effects of chemotherapy, control mice bearing no tumors (NTC) also received 3 chemotherapy treatments beginning 16 days following tumor vehicle injection. Survival probability was significantly increased in mice in IG (47%) compared to GI (19%) groups due to greater inhibition of tumor growth. The number of deaths due to the toxicity of chemotherapy was similar between groups. This animal tumor model should aid in elucidating mechanisms that may govern the responsiveness of tumors to chemotherapy and the variability of chemotherapeutic efficacy in human cancer patients.

Supported by the Medical Research Council of Canada, and a University Graduate Fellowship from UBC.

639.4

PROLONGED TREATMENT OF PERTUSSIS TOXIN AFFECTS MORPHINE'S ACTION ON TIDA NEURON ACTIVITY AND PROLACTIN SECRETION. J.T. Pan* and J.Y. Lin, Dept. Physiol., Natl. Yang-Ming Univ., Taipei, Taiwan, R.O.C.

The effects of pertussis toxin (PTX) pretreatment on basal and morphine-affected changes of tuberoinfundibular dopaminergic (TIDA) neuron activity and serum prolactin level in ovariectomized Sprague-Dawley rats treated with a long-acting estrogen (polyestradiol phosphate, 0.1 mg/rat, sc) were assessed in the study. The activity of TIDA neurons was determined by measuring the turnover rate of dopamine, the concentrations of DOPAC or the accumulation of DOPA in the median eminence. Acute (30-90 min) treatment of PTX (0.025-2.5 μg/rat, icv) had no significant effect on any of the parameters measured. Prolonged (24 h) treatment of PTX (1 μg/rat, icv), while had no effect on basal TIDA neuron activity either, significantly reduced morphine's (10 mg/kg BW, sc) inhibitory effect on TIDA neuron activity (using DOPA, but not DOPAC as the index), and stimulatory effect on PRL release. The dopamine levels in the median eminence, however, were significantly reduced by prolonged treatment of PTX, indicating a reduction of TIDA neurons. These results suggest that PTX-sensitive GTP-binding proteins may be responsible for the maintenance of TIDA neurons, and for mediation of the inhibitory effect of morphine on TIDA neuron activity, and in turn, the stimulation of prolactin secretion.

639.5

ONTOGENY OF ADRENAL NITRIC OXIDE SYNTHASE ACTIVITY AND RESPONSE TO ENDOTOXIN. J.R. Tobin* and L.C. Moore. Department of Anesthesia, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1009

Nitric oxide (NO) acts as an inhibitory neurotransmitter in the autonomic nervous system and has been isolated in the adrenal medulla and cortex. In the adrenal cortex NO inhibits steroid secretion. Developmental differences in sympathoadrenal responses to stress and endotoxemia have been clinically noted with early fatigue in juveniles. We examined the ontogeny of nitric oxide synthase (NOS) activity in rat adrenal, the adrenal NOS response to endotoxin, and effect of treatment with systemic dexamethasone.

Wistar Kyoto rats of four age groups were studied (<48 hrs, 2, 4, and 12 wks of age [n=8/group]). Animals were anesthetized with pentobarbital ip and adrenals were harvested, homogenized and centrifuged (10000*g for 15 min at 4°C). In a second experiment, endotoxin (1 mg/kg ip) or saline was administered to 4 and 12 week old rats (n=8/group) 4 hr prior to harvest. A third cohort (n=8/group) of 8 week old rats received saline or dexamethasone (0.2 or 2.0 mg/kg ip daily) for 1 week. NOS activity was assayed in supernatant cytosolic fractions by conversion of [^{14}C]-arginine to [^{14}C]-citrulline. Data were analyzed by ANOVA or the Kruskal-Wallis test with $p<0.05$ considered significant (*).

NOS activity in adrenals is greatest in newborn rat pups and declines with age (<48 hr: 9.04±1.51, 2 wk: 7.39±0.8, 4 wk: 5.68±0.48*, 12 wk 3.98±0.50* pmol/mg prot/min [mean ±S.E.]). The 12 week group was statistically different than all other age groups. Endotoxin elicits an increase in NOS activity in 4 week old rats: 22.1±4.3* vs saline control 6.0±0.5, but no increase in 12 week old rats: 3.89±0.62 vs saline 2.98±0.3 pmol/mg prot/min. Western blotting demonstrated increased expression of NOS protein. Dexamethasone causes a dose-dependent decrease in adrenal NOS : saline 4.37±0.5, 0.2 mg/kg 3.21±0.53, 2.0 mg/kg 1.95±0.28* pmol/mg prot/min.

Age related differences in sympathoadrenal responses to stress are correlated with developmental differences in adrenal NOS expression. As NO appears to act as an inhibitory neurotransmitter in the adrenal, age related differences in NOS expression may partially explain juvenile sympathoadrenal fatigue during stress and endotoxemia. Dexamethasone elicits dose dependent adrenal atrophy and decreased adrenal NOS.

639.6

NITRIC OXIDE SYNTHASE SUBTYPE I AND SUBTYPE III IN THE PINEAL GLAND OF THE SHEEP: AN EXPERIMENTAL IMMUNOHISTOCHEMICAL STUDY. **M.O. LÓPEZ-FIGUEROA[1], J.P. RAVAULT[2], B. COZZI[3]* AND M. MØLLER[1]** [1] Institute of Medical Anatomy, University of Copenhagen, DK-2200 Copenhagen, Denmark; [2] Institute of Anatomy of Domestic Animals, University of Milan, I-20133 Milan, Italy; [3] Institute of Comparative Physiology, University of Tours, F-37200 Tours, France.

By use of immunohistochemistry, nerve fibres immunoreactive to subtype I (neuronal) of the enzyme nitric oxide synthase (NOS) were demonstrated in the pineal gland of sheep. The NOS-immunoreactive fibres were located in the pineal capsule and the connective tissue septae of the gland, but nerve fibres were also present adjacent to blood vessels and intraparenchymally between the pinealocytes.

By use of an antibody directed against subtype III (endothelial), only the endothelium of pineal blood vessels was stained. The pinealocytes of the sheep were not stained neither by the antibody against subtype I nor subtype III.

In order to trace the origin of the NOS-immunoreactive nerve fibres, a series of sheep had both superior cervical ganglia removed. NOS nerve fibres were still present in the gland one month after the ganglionectomy. Also the endothelial NOS-immunoreactivity was present one month after the bilateral superior cervical ganglionectomy.

Thus, our data show a prominent innervation of the sheep pineal gland with nitric oxide synthase immunoreactive fibres with origin outside the sympathetic nervous system. This indicates that the sheep pinealocyte metabolism and the regulation of the circulation might be influenced by nitric oxide originating both in non-sympathetic nerve fibres as well as by nitric oxide with origin in the endothelium of the blood vessels. The nitric oxide, located presynaptic in the nerve fibres, might also regulate release of neuropeptides located in the same fibres.

639.7

THE USE OF THE NEUROTROPIC VIRUS PSEUDORABIES TO DEMONSTRATE CNS PATHWAYS TO THE RAT FOOTPAD - AN EXTENDED VIEW OF THE ATONOMIC NEURAXIS. B.J. Oldfield* and R. Miselis+. Howard Florey Institute, University of Melbourne, Australia 3052 and University of Philadelphia, PA 19104+

The innervation of the footpad has been utilised recently as a model for the chemical coding of defined autonomic pathways. The aim of this study is to use the transynaptic transfer of psuedorabies virus (PRV) to map the distribution of chains of connected neurons directed to sudomotor and vasomotor endpoints in the footpad. Thirty four male Sprague Dawley rats were anaesthetised with sodium pentobarbitone (60 mg/kg i.p.) and 0.5 µl of a solution containing PRV was injected into several sites in the left forepaw. After survival periods ranging between 24 and 100 hours, rats were reanaesthetised and perfused transcardially with 4% paraformaldehyde. Frozen sections were exposed to antisera raised against PRV as well as tyrosine hydroxylase (TH) and a range of peptides. Infected neurons were first detected in the ipsilateral stellate ganglion 40 hrs. after inoculation. Infected neurons included populations, which were TH-positive and TH-negative (cholinergic, approx 30%). Over the next 50 hrs. infected neurons were found first in the sympathetic preganglionic cell groups of the upper thoracic spinal cord then coincidentally in the rostroventrolateral medulla and parvocellular paraventricular nucleus (PVN). Up to 50% of the neurons in the medulla were catecholaminergic whereas infected neurons in the PVN were not vasopressin positive and less than 1% were colocalised with oxytocin. Subsequently neurons were infected in the lamina terminalis, bed nucleus of the stria terminalis and preoptic area. Despite a general codistribution of the latter neurons with neurons containing atrial natriuretic peptide (ANP) none were found to the ANP-positive. These results demonstrate discrete populations of neurons that are directed to the footpad and provide the basis for neurochemical coding of these pathways.

This work was supported by grants from the NHMRC, Australia.

639.8

INFLUENCE OF SYMPATHETIC DENERVATION OF THE EYE ON OUTFLOW OF THE INTRAOCULAR FLUID. M.I.Rasumovskij*, N.V.Balashov, A.R.Grigorian, Institute of Occupational Guidance, Lab. of Vision Physiology, 195067, St.Petersburg, Russia.

In experiments on anaesthetized cats the effects of sympathetic denervation of the eye on the outflow of the intraocular fluid, which is one of the main factors of the level of intraocular pressure, have been investigated. The accuracy of the eye sympathetic denervation was controlled morphologically and by the level of adrenaline in the iris and cyliary body. The results clearly demonstrate that the level of the background outflow of the intraocular fluid was practically the same both in control and sympathectomized groups of animals. When the intraocular pressure was rised step by step the sharp increasing of the outflow of intraocular fluid in denervated eye was observed. At the same time the visible decreasing of noradrenaline in eye tissue was marked. These findings suggested that postganglionic sympathetic fibres exert direct regulatory action on the eye vasomotor tones and drainage apparatus and any disturbances in this system can lead to loss adaptive functions supporting intraocular pressure on normal level.

639.9

PROADRENOMEDULLIN N-TERMINAL 20 PEPTIDE (PAMP) REDUCES NICOTINE-INDUCED CURRENTS IN BOVINE ADRENAL CHROMAFFIN CELLS. I. Shibuya, T. Nagatomo, N. Kabashima, Y. Ueta, Y. Toyohira,[1] Y. Uezono,[1] N. Yanagihara,[1] A. Wada,[2] F. Izumi[1] & H. Yamashita* Depts. of Physiol. and Pharm.,[1] Univ. of Occup. and Environ. Health, Kitakyushu 807, Japan and Dept. of Pharm.,[2] Miyazaki Medical collage, Miyazaki, 880, Japan

Proadrenomedullin N-terminal 20 peptide (PAMP), a novel peptide processed from the adrenomedullin precurser, is co-secreted from adrenal medulla with adrenomedullin as well as catecholamines. Both PAMP and adrenomedullin were shown to have hypotensive effects (FEBS Lett. 351, 1994). Recently, it has been reported that PAMP reduces catecholamine secretion induced by the nicotinic receptor stimulation (J. Neurochem., 64, 1995). To elucidate the anticholinergic mechanisms of PAMP, we recorded nicotine-induced currents from bovine adrenal chromaffin cells and analyzed the effects of PAMP on the currents by using the whole-cell patch-clamp technique. PAMP alone (10^{-6}-10^{-9} M) did not induce ionic currents at a holding potential of -80 mV, however, it dose-dependently reduced inward currents induced by nicotine (Nic, 10^{-5} M). The inhibition remained for about 10 min after washout of PAMP. When applied during Nic stimulation, PAMP accelerated the desensitization process. These results suggest that PAMP inhibits nicotinic currents by increasing the rate of desensitization and that PAMP may play a role as an anti-hypertensive hormone by inhibiting catecholamine secretion via autocrine and/or paracrine.

639.10

INNERVATION PATTERN OF SYMPATHETIC PREGANGLIONIC NEURONS PROJECTING TO SUPERIOR CERVICAL AND STELLATE GANGLIA. K. Asamoto*, N. Tamamaki and Y. Nojyo., Dept. of Anatomy, Fukui Medical School, Matsuoka, Fukui 910-11, Japan.

Anterograde labeling technique with PHA-L was employed to observe the arborization pattern of a single preganglionic axon in the superior cervical ganglion (SCG) and stellate ganglion (STG) of rats. We drew three preganglionic axons in both SCG and STG in their full length. In SCG, the labeled axon extended for 600-700 µm in the rostrocaudal and about 200 µm in the transverse direction. Terminal twigs of each preganglionic axon surrounded 11, 14 and 11 postganglionic neurons, respectively. In STG, the extent of axonal arborization was 400-800 µm in the rostrocaudal and 400 µm in the transverse direction. These axons surrounded at least 21, 19 and 20 postganglionic neurons, respectively. We also observed the segmental relationship between upper thoracic spinal cord and cervical sympathetic ganglion detecting c-fos like protein in postganglionic neurons after electrical stimulation of ventral roots at each spinal segment. We could observe clear segmental relationship between spinal cord and STG, but not between spinal cord and SCG.

639.11

EFFECT OF PARATHYROID HORMONE AND CALCITONIN ON CHOLINERGIC NEUROTRANSMISSION IN RAT PARATHRYOID GLANDS D.P.Cardinali*, J.E.Stern, Depto. de Fisiologia, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

We have shown in previous studies that the activity of sympathetic and parasympathetic nerves in the cervical region affect parathyroid hormone (PTH) and calcitonin secretion. Additionally, cholinergic neurotransmission in superior cervical ganglion (a site of origin of the sympathetic innervation to the thyroid-parathyroid territory) was significantly modified by calciotropic hormones. To further substantiate the hypothesis of the existence of a feedback loop in neural regulation of calcium homeostatic hormones, the effect of PTH and calcitonin on ^{3}H-choline uptake and on ^{3}H-acetylcholine synthesis by rat parathyroid glands were examined. Choline uptake synthesis was measured by incubating freshly dissected parathyroid glands with ^{3}H-choline in the presence or absence of hemicolinium-3 (a specific inhibitor of the high affinity carrier of choline). Acetylcholine synthesis was assayed by the conversion of ^{3}H-choline to ^{3}H-acetylcholine. Incubation of tissue with PTH or calcitonin resulted in a bell-shaped, dose-dependent inhibition of ^{3}H-choline uptake. The effect of PTH was blunted by preincubation with NLe8-18-PTH (3-34) amide (a specific PTH antagonist). PTH induced a dose-dependent inhibition of ^{3}H-acetylcholine synthesis, effect also blocked by the specific peptide antagonist. Calcitonin was effective to block ^{3}H-acetylcholine synthesis only at a concentration of 10^{-7}M. This results indicate that in vitro, PTH and to a less extent calcitonin, inhibit cholinergic activity in rat parathyroid glands. In view of the inhibitory effect that parasympathetic terminals have on PTH secretion, this feedback mechanism could facilitate a fast release of PTH upon a hypocalcemic challenge

639.12

SYMPATHETIC STIMULATION ACCELERATES WOUND HEALING AT THE EPIDERMAL AND DERMAL LEVELS OF SKIN L.R. Kim and B.H. Pomeranz* Departments of Physiology and Zoology, University of Toronto, Toronto, ON, Canada M5S 1A1

Neurogenic inflammation is mediated by unmyelinated sensory afferents and sympathetic efferents. Although inflammation is the initial stage of wound healing, the role of nerves in wound healing is not well-established. In a recent study we demonstrated that sympathetic post-ganglionic neurons (SPGNs) were necessary for normal rates of cutaneous wound healing since sympathetic denervation significantly attenuated the rate of wound healing.

The present study further elucidates the role of SPGNs in cutaneous wound healing. After stimulation of the SPGNs using 0.2mg/kg of 6-hydroxydopamine (6-OHDA) applied locally during the inflammatory period, we report a 43% increase in breaking strength, a measure of dermal collagen re-establishment ($p<0.05$, $n_e=13$, $n_c=10$, one way ANOVA). Furthermore, a 38% increase was found in the rate of epidermal wound healing as measured by transcutaneous electrical resistance (TER) ($p<0.05$, $n_e=21$, $n_c=18$, one way ANOVA). This new method we have developed has been histologically shown to demonstrate the re-establishment of the ion-resistant rat epidermis. Control animals were given the vehicle solution, 1% ascorbic acid. These results show that chemical stimulation of SPGNs markedly accelerates skin wound healing at both the epidermal and dermal levels. These are the first studies to show a role for SPGNs in the regulation of wound healing. It has been suggested that the SPGNs promote neurogenic inflammation and thus wound healing by the secretion of NA, ATP, and the *de novo* synthesis of prostaglandins at the sympathetic terminal.

639.13

ADRENOCORTICAL SYMPATHETIC CO-CULTURES: A MODEL FOR THE STUDY OF NEURAL-ADRENOCORTICAL INTERACTION. J.L. Buberel*, M.A. Holzwarth, Neuroscience Program and Department of Physiology, University of Illinois, Urbana, Illinois, 61801.

In order to study the sympatho-adrenal interactions of the adrenal cortex, we have established co-cultures of adrenocortical and sympathetic ganglion cells. Sympathetic neurons from sympathetic chain ganglia of 4-6 day old rat pups are grown in supplemented DME/F12 medium and dissociated adrenocortical (AC) cells from adult rats are added at various times. We have used immunocytochemistry (ICC) to characterize the cell cultures and to study the relationships between the sympathetic neurons and AC cells. The sympathetic neurons usually cluster in groups of 2-10, and begin to extend neurites within 24 hours. Neurite length can exceed 5 mm, and neurites often form fascicles after 4 days. These sympathetic neurons, identified by 2H3 ICC, continue to express tyrosine hydroxylase and dopamine-β-hydroxylase. A subpopulation are also immunoreactive for VIP. We have used synaptophysin ICC as a marker for neuronal maturation and potential for functional autonomic synapse formation. Synaptophysin is detectable after 2 days, and forms puncta along neurites and cell bodies after 4 days, suggesting the formation of presynaptic release sites. AC cells are generally rounded, contain many lipid droplets, express 3-β-hydroxysteroid dehydrogenase, and secrete steroids. The addition of AC cells to sympathetic cultures at days 2, 7, and 14 does not appear to affect neurite extension or the expression of 2H3, dopamine-β-hydroxylase, tyrosine hydroxylase, or synaptophysin in the sympathetic neurons. The neurites grow close to, over, and around the AC cells. Thus, neither the presence of the AC cells nor their secreted products, appear to inhibit the growth of the neurites from the sympathetic neurons. The close morphological relationship attained in vitro suggests that this model is a useful tool for the study of the sympathetic control of AC cellular functions.

639.14

PRIMARY CO-CULTURE OF CORTICOSTEROIDOGENIC AND CHROMAFFIN CELLS FROM THE FROG INTERRENAL GLAND AS A MODEL OF MEDULLARY CONTROL OF STEROIDOGENESIS.
S.L. Shepherd* and M.A. Holzwarth. Neuroscience Program and Department of Physiology, University of Illinois, Urbana, IL 61801

Recent studies show that adrenocortical cell secretion is modulated by adrenal medullary cells, and hence, indirectly by splanchnic nerve activity. We have chosen the frog adrenal as a model to study the chromaffin cell modulation of adrenocortical secretion because chromaffin and adrenocortical cells are interspersed. Also, the frog chromaffin cells, like a subpopulation of their mammalian counterparts, extend processes which might allow direct interactions between corticosteroidogenic and chromaffin cells. To facilitate the investigation of adrenal cell interactions, we have established and characterized primary cell co-cultures of frog adrenal cells. The corticosteroidogenic cells have a low basal steroid secretion rate ($1.4x10^{-5}$ng/cell/24hr corticosterone (B), $9.7x10^{-5}$ng/cell/24hr aldosterone (aldo)) and respond to 10^{-8}M ACTH with an increase in B (15-fold) and aldo (5.3-fold) secretion. Chromaffin cells cultured in the presence of adrenocortical cells extend unbranched processes of 50 to 300 μm which often abut upon or overlie adrenocortical cells. The chromaffin cells continue to express tyrosine hydroxylase, PNMT, dopamine ß-hydroxylase, and enkephalin and are also immunoreactive for NCAM and linc (amphibian neural cell marker). Techniques for demonstrating transmitter release from chromaffin cells and adrenocortical steroidogenesis at the level of single cells are being optimized. We use indirect immunofluorescence of dopamine ß-hydroxylase, a chromaffin granule membrane antigen, to show neurotransmitter release from chromaffin cells elicited by 10 minute stimulation with 2 mM carbamylcholine or 55 mM potassium. Activation of adrenocortical cells following 30 minute stimulation with 2 mM carbamylcholine or 10^{-8}M ACTH is shown by Fos. These techniques will be used to test the hypothesis that chromaffin cell activity directly modulates steroidogenesis.

639.15

DRINKING AND BLOOD PRESSURE RESPONSES TO CENTRAL INJECTION OF L-NAME IN CONSCIOUS RATS. H. Liu, M.L. Terrell, J.Y. Summy-Long†, M. Kadekaro*. Division of Neurosurgery, UTMB, Galveston, TX and †Department of Pharmacology, Hershey Medical Center, Pennsylvania State University, Hershey, PA.

We studied the effect of N^G-nitro-L-arginine methyl esther (L-NAME), a potent blocker of nitric oxide (NO) synthase, on water intake and blood pressure (BP) responses in conscious male albino rats during osmotic stimulation and hemorrhage. Thirty min after injection of 1.0 M NaCl (15 ml/kg, s.c.) or 10 min after the beginning of hemorrhage (0.7 ml/min to a 20% blood volume loss), artificial cerebrospinal fluid (aCSF) or L-NAME (10, 250 or 500ug/5μl) were injected icv. Ten min later, water was presented to rats and its cumulative intake was measured for 2h. During osmotic stimulation and hypovolemia, L-NAME at doses of 250 and 500μg, but not 10μg, attenuated ($p<0.05$) water intake compared to the control group. Mean arterial BP, which increased 30 min after osmotic stimulation ($p<0.05$), remained at pressor levels after 250 and 500μg of L-NAME, but decreased progressively below basal levels after treatment with aCSF and the lowest dose of L-NAME (10μg). Ten min after starting hemorrhage, BP fell ~ 60 mm Hg ($p<0.05$) in all groups but increased after hemorrhage ceased. The fall in BP associated with hemorrhage returned to control levels after treatment with 250 and 500μg of L-NAME but not after aCSF or 10μg of L-NAME. These results indicate that NO is involved in the regulation of drinking behavior and may have an important role in the central control of BP during osmotic stimulation and hypotensive hemorrhage. (Supported by NIH grants NSR01-23055 to MK and HDR01-25498 to JYSL.)

639.16

FLAVONES ANTAGONIZE LIPOPOLYSACCHARIDE-INDUCED SICKNESS IN MICE. R. J. Fishkin, R. Corbett* and J. T. Winslow.
Hoechst-Roussel Pharmaceuticals, Inc.
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Previous studies indicate that indomethacin, methylprednisolone and an interleukin-1 receptor antagonist inhibit lipopolysaccharide (LPS)-induced sickness behavior in rats. In the current studies we replicated and extended these findings in the mouse to a novel class of anti-inflammatory drugs: flavones. A relatively low dose of LPS (15 μg/kg, ip) consistently induced a 50% reduction in the social investigation of a juvenile conspecific by an adult male mouse. Pretreatment with methylprednisolone (30 mg/kg, ip), indomethacin (3-10 mg/kg, ip), and ibuprofen (10 mg/kg, ip), but not tenidap (1-100 mg/kg, ip) 1 hr before LPS injection completely blocked LPS-induced sickness behavior. Dexamethasone (0.1 mg/kg, ip) significantly attenuated LPS-induced sickness behavior. The flavones L82-05971 (0.25 mg/kg, ip), L86-8276 (0.03 mg/kg, ip) and chrysin (10 mg/kg, ip) also antagonized LPS-induced sickness. The psycho-motor stimulant amphetamine (2-4 mg/kg, ip) did not relieve, and actually exacerbated LPS-induced decreases in investigation. This model may provide a sensitive method of identifying compounds which selectively antagonize behavioral effects associated with interleukin release.

639.17

Spatial organization and representation are impaired in Cushing's disease. **H. Forget[1], H. Cohen[1]*, M. Somma[2], and A. Lacroix[3]. [1]Laboratoire de neuroscience de la cognition, Université du Québec, Montréal, Qc, H3C 3P8, Canada; [2]Service d'Endocrinologie, Hôpital Notre-Dame et [3]Service d'Endocrinologie, Hôpital Hôtel-Dieu, Université de Montréal, Qc, H2L 4K8, Canada.**

It is now well established that the brain is a major target for glucocorticoid hormones and that cognition and emotion are affected by inadequate levels of circulating hormones. The hippocampus is a crucial structure as it is the richest in glucocorticoid-binding receptors and is thus particularly vulnerable to glucocorticoid excess. In this study, we tested whether an excess of glucocorticoid hormones has an effect on the treatment of spatial organization and representation. In the animal litterature, both location and identity of an object depend upon hippocampal integrity. In this perspective, we compared the spatial organization and representation (line orientation, visual organisation, visual recognition, Block Design, Object assembly, Picture completion) of 12 patients with chronic hypercortisolemia due to Cushing'disease (CD) to that of healthy control subjects yoked for age, sex and education (mean age= 45.2±9.4 yrs; mean education= 12.3±9.3 yrs). MANOVA results showed a general impairment in spatial tasks (Pillais=.5511, $F_{1,21}$= 3.27,p=.027) and, except for visual recognition, subsequent univariate F tests showed group differences in all tasks (all p's < .05). These results show that impairment in the treatment of spatial information may be associated with elevated cortisol levels in patients with CD. It remains to be established whether the observed deficits in the treatment of spatial information, in CD patients, occur at a perceptual or at a processing/treatment level.

639.18

DOPAMINE MODULATION OF GABA NEUROTRANSMISSION IN HYPOTHALAMIC ARCUATE NUCLEUS NEURONS. A.B. Belousov* and A.N. van den Pol. Dept. Biology, Stanford Univ., Stanford, CA 94305 and Sect. Neurosurgery, Yale Univ., New Haven, CT 06520.

Dopamine (DA) neurons and their terminals are widely distributed within the hypothalamus and DA plays an important role in the regulation of the hypothalamic neuroendocrine system. In our previous work, DA (30 μM) was found to decrease glutamate neurotransmission. This DA effect was partly due to a decrease in the amplitude and frequency of excitatory post-synaptic potentials (EPSPs), and could also be due to a DA-mediated increase in GABAergic activity. To explore the hypothesis that activation of GABAergic neurons might be one of the mechanisms of DA's inhibitory effect on glutamate activity we used whole cell patch clamp recording of neurons (n=49) cultured from the hypothalamus or arcuate nucleus. In current clamped neurons (average membrane potential -60 mV), DA application (10-200 μM) caused an increase in the frequency of spontaneous GABA-mediated IPSPs in the majority of the tested cells (10 of 11). In cells held at -25 mV, DA (10 μM) evoked the appearance of inhibitory postsynaptic currents (IPSCs) in 4 of 5 cells and significantly increased the amplitude (3 of 11 cells; by 150%+) and frequency (9 of 11; 300%+) of spontaneous IPSCs. The spontaneous and DA-evoked IPSCs were blocked by 50 μM bicuculline, verifying their GABAergic origin. This finding together with data showing that DA had no effect on the input resistance suggested that the observed DA effects were at least partly achieved through the potentiation of GABA release.

639.19

CALCIUM REGULATION OF HYPOTHALAMIC FOS GENE EXPRESSION FOLLOWING DEPOLARIZATION D.M. Witt* and H. Gainer, Lab. of Neurochemistry, NINDS, NIH Bethesda, MD 20892.

The complexities of the *in vivo* environment in the brain make it difficult to determine the signal transduction mechanisms involved in stimulus-coupled neuropeptide gene expression. Therefore, we have developed an *in vitro* slice explant model for examining the effects of neurotransmitter / neurosteroid stimulation of neurons in a controlled environment. Using electrophysiological and organotypic slice explant technologies, acute slice explants were used to study Fos expression in the paraventricular nucleus of the hypothalamus (PVN) derived from postnatal rats. In 1.5 hrs of stimulation *in vitro* , either K^+ (40 mM), glutamate (100 μM) or veratridine (1 μM) depolarization produced varying degrees and topographically distinct patterns of Fos expression in magnocellular, parvocellular and periventricular regions of the PVN as well as the suprachiasmatic nucleus. The role of calcium in Fos gene expression, using the above depolarization paradigms, was examined either in the absence of extracellular calcium or by pretreating the acute slice explants with N- channel or L-channel calcium blockers. In the absence of extracellular calcium, Fos induction did not occur after depolarization. The L-channel dihydropyridine (Nifedipine, 1 μM) was most effective in inhibiting depolarization induced Fos expression. In contrast, the N-channel blocker (ω -conotoxin, 1 μM) had little or no effect on Fos expression. These data strongly implicate calcium influx, via L-channels, as the principal mechanism of depolarization-induced Fos gene expression in neurons in the mammalian hypothalamus and suggests that this experimental preparation will be very useful in exploring the regulation of other immediate early genes, and neurotransmitter / neuromodulatory influences.

GASTROINTESTINAL REGULATION: GASTROESOPHAGEAL CONTROL

640.1

THE EXISTENCE OF NEURONS IN THE PARAVENTRICULAR NUCLEUS(PVN) PROJECTING TO BOTH POSTERIOR PITUITARY AND STOMACH. B.H. Lee*, W.S. Choi, K.J. Cho, M. O. Kim, H.J. Sohn[1], R.R. Miselis[2], S.H. Baik[1]. Dept. of Anatomy, Medical School, Gyeongsang Nat'l Univ., [1]Dept. of Anatomy, Medical School, Seoul Nat'l Univ., Republic of Korea, and [2]Inst. of Neurol. Sci., Univ. of Pennsylvania, Philadelphia, PA19104.

The efferent fibers from magnocellular and parvicellular subunits of the PVN have been known to subserve separate roles in posterior pituitary and brainstem nuclei supplying the vagus nerve (neuroendocrine and autonomic functions, respectively). To investigate the neurons projecting to both neuroendocrine and autonomic nervous systems especially projecting to the stomach, viral and CT-HRP retrograde transneuronal labelling methods were used. Pseudorabies virus(PRV) was injected into the stomach wall of the adult Sprague-Dawley rats. After 2 days, the rats were given another injection of CT-HRP into the posterior pituitary with the aid of stereotaxic frame . After totals of 3 ~ 5 days of survival, rats were perfused with 4% PLP and their brains were processed for triple detection of PRV, CT-HRP or oxytocin. In longer surviving groups, some cells in the lateral magnocellular group of PVN throughout the whole brain areas showed triple immunoreactivity. In conclusion, this study demonstrates that some of the CNS oxytocinergic cells and gastric motor neurons share their origins from the same cell.

640.2

BICUCULLINE IN THE DORSOMEDIAL HYPOTHALAMIC NUCLEUS INCREASES INTRAGASTRIC PRESSURE IN RATS. D.V.Sivarao, A.Van Bergen and P.J.Hornby*. Department of Pharmacology, LSUMC. New Orleans, LA 70112.

Blockade of GABAergic tone in the dorsomedial hypothalamus (DMH) increases intestinal motility (Greenwood and DiMicco, Am.J.Physiol. 268:G514-G521, 1995). Hypothesis: Microinjection of bicuculline methiodide (BMI) into the DMH increases gastric motor function. Methods: BMI (30 pmol in 30 nl) or vehicle were microinjected into the DMH of αchloralose-anesthetized rats. Intragastric pressure (IGP, cmH_2O), pyloric minute motility index (PMMI), mean blood pressure (BP, mmHg) and heart rate (HR, bpm) were compared before and after microinjection by student's t-test. Results:

	DMH		NEAR DMH	OUTSIDE DMH
	Δsaline(N=3)	ΔBMI(N=6)	ΔBMI (N=6)	ΔBMI (N=18)
Peak IGP	0.0±0.0 [1]	1.4±0.1*	0.4±0.1	0.1±4.9
PMMI	0.0±0.0	-0.8±0.8	-0.3±1.1	0.1±6.1
BP	-3±2	28±5*	25±3*	3±2
HR	-1±1	67±13*	51±8*	10±5

[1]Mean±SEM; * P<0.05 compared to saline control. Atropine (0.5mg/kg i.v.) abolished the effect of BMI microinjection into DMH on IGP (0.4±0.1) but not on BP (19±7) and HR (48±10). Conclusions: BMI microinjection into DMH, but not into the surrounding areas, significantly increases IGP through a vagally-mediated pathway. Thus, modulation of resident GABAergic tone in this hypothalamic nucleus may contribute to stress-evoked effects on gastric motor function. Supported by PHS grant DK 42714 and the A.P. Sloan Foundation.

640.3

EVIDENCE OF THE INVOLVEMENT OF GASTRIC VAGAL AFFERENTS ESOPHAGEAL COLLATERAL IN A REFLEX ACTION: SHORT VAGO-VAGAL REFLEX.
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Esophageal distension (ED) has been shown to induce gastric relaxation, and vagal cooling does not completely abolish this effect (Dig Dis Sci 34:873-881, 1989). We have reported that gastric vagal afferents may emit esophageal collateral branches before reaching the stomach (J Auton Nerv Syst 1995, in press). Aim: using an isolated gastroesophagus in vivo preparation, to determine if short-term lower esophageal distension (ED) can alter intragastric pressure (IGP). Overnight fasted, urethane anesthetized rats were used. Three ligatures were made at cervical esophagus, pylorus and gastroesophageal junction but the gastric vagus nerve was kept intact. A double-lumen catheter was inserted into the stomach for infusing, draining saline and measuring IGP. The basal IGP was kept at 4 to 5 cm H_2O. A catheter with latex balloon was placed at lower thoracic esophagus and distended with a bolus of saline for ~ 5 sec. The IGP (Mean ± SEM cm H_2O) was decreased 0.74 ± 0.09 (N=8) for 0.5 ml ED; 0.51 ± 0.04 (N=10), 0.50 ± 0.08 (N=8) and 0.42 ± 0.08 (N=7) for 0.4, 0.3 and 0.2 ml ED, respectively ($p<0.05$ ANOVA). Bilateral vagotomy reduced the effect of 0.5 ml ED from 0.74 ± 0.09 to 0.36 ± 0.01 (N=4), whereas a further bilateral splanchnicotomy rebounded, instead to abolishing, the effect of 0.5 ml ED from 0.36 ± 0.01 to 0.45 ± 0.03 (N=3) ($p< 0.05$, ANOVA) indicating the splanchnic nerve provided a tonic excitatory influence to IGP. These results indicate that a shot-term ED can induce gastric relaxation. About 50% of this effect is CNS-dependent, probably via long vago-vagal reflex, whereas the other 50% can be maintained without CNS and ENS involvement, probably via proposed gastric vagal-esophageal collateral reflex arc, short vago-vagal reflex. (Supported by NIH Grants NS 28433 & DK30110)

640.4

PYY-CONTAINING NEURONS AND TERMINALS IN THE NUCLEI RELATED TO THE AUTONOMIC REGULATION IN RATS. H. Yang*, L. Wang, H. Wong, and Y. Taché. CURE. VA Medical Center. Brain Research Institute and Dept. of Medicine, UCLA. Los Angeles, CA 90073, U.S.A.

We previously reported that microinjection of PYY into medullary nuclei related to the vagal regulation stimulated gastric acid secretion (AJP 268, 1995). In this study, using a specific polyclonal antibody against PYY (CURE antibody Core, #9152), immunohistochemistry was performed to examine the location of PYY-positive neurons and terminals in the nuclei related to the autonomic regulation in colchicine treated rats. In the medulla, high density of PYY-immunoreactive nerve terminals and fibers were observed in the dorsal vagal complex and raphe pallidus throughout their caudal to rostral extension, while moderately dense networks were found in the area postrema, raphe obscurus (Rob), nucleus ambiguus and the nearby reticular formation. A group of large cells were located in the ventrolateral medulla area throughout the same caudal-rostral extension as the Rob. The cells in the caudal part of this cell column were a dense group but gradually became scatter as it extended rostrally. A few PYY-positive cells were observed in the rostral part of the nucleus solitary tract. Another large group of neurons were located in the caudal pons at the segment between the facial nerve and the Rob. These were small cells scattered within 1 mm in both sides around the midline. A moderate terminal network and a few cells were located in the locus coeruleus in the pons. PYY-containing fibers formed a loose distributed network in the periaqueductal gray in the midbrain. In the hypothalamus, dense PYY-positive terminals were located in the paraventricular nucleus and arcuate nucleus while moderate dense fibers were observed in the lateral hypothalamus, supraoptic nucleus and median eminence. A few cells were observed in the arcuate nucleus and the area dorsolateral to the ventromedial hypothalamic nucleus. Small PYY-positive cells and fibers also scattered in the amygdaloid nuclei throughout its caudal-rostral extension. These results indicate that centrally originated endogenous PYY may play an important role in the autonomic regulation.

640.5

BOMBESIN-LIKE IMMUNOREACTIVE (LI) NERVE TERMINALS IN THE DORSAL VAGAL COMPLEX (DVC) IN RAT, AN ELECTRON MICROSCOPIC ANALYSIS. Richard B. Lynn*[1], Lesley S. Bechtold[1], Richard R. Miselis[2]; [1]Dept. of Medicine, Thomas Jefferson Univ., Philadelphia, PA 19107; [2]Dept. of Animal Biology, Univ. of Pennsylvania, Philadelphia, PA 19104

Bombesin inhibits gastric function and induces satiety when microinjected into the DVC. At the light level bombesin is in apparent nerve terminals in the DVC which includes the dorsal motor nucleus of the vagus (DMV) and the nucleus of the solitary tract (NTS). Electron microscopy was used to determine if synaptic contacts were present. We used a preembedding immunoperoxidase technique with bombesin antiserum (Incstar). Frequent thin sections (0.35μ) were stained with toluidine blue to identify anatomic sites. Ultrathin sections were examined using a Zeiss 10 TEM. In the DMV, bombesin-LI nerve terminals typically contacted small to medium sized dendrites (0.9-1.7μ). Occasional bombesin-LI terminals were associated with a full range of postsynaptic structures including large (proximal) dendrites, small dendrites, perikarya and rarely axon terminals. Of 65 axo-dendritic contacts studied in the DMV there were 38 asymmetric and 15 symmetric synapses, and 12 nonsynaptic close apositions. Similar to other neuropeptides, the bombesin-LI reaction product was predominantly over dense core vesicles located in regions distant from the synaptic junction. Bombesin-LI nerve terminals in the NTS have a similar profile of contacts. Bombesin containing nerve terminals in the DVC form mostly axo-dendritic contacts. This study provides further evidence that bombesin is a neurotransmitter/ neuromodulator in the DVC, potentially effecting vagal motoneurons. Support: DK02094(RL); GM27739(RM)

640.6

SEROTONIN AUGMENTS TRH ANALOGUE-STIMULATED GASTRIC AID SECRETION BUT NOT ANTRAL MOTILITY AFTER MICROINJECTION INTO THE DORSAL VAGAL COMPLEX (DVC).

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TRH and serotonin (5-HT) interact at the DVC to augment gastric acid output (GAO) and erosions. It is unclear whether the TRH/5-HT interaction at the DVC extends to gastric motility. Urethane anesthetized 24-hr fasted rats were equipped with a gastric fistula for the acid studies or serosal antral strain gauges for the motility study. 5-HT (49 pmol) produced a 100% increase in stable TRH analogue RX77368 (RX; 4pmol)-induced stimulation of GAO after DVC co-microinjection (10nl) [mean ± SEM , acid output (μ mole/1hr) RX: 37 ± 3 ; RX + 5-HT: 74 ± 10; p< 0.05] By contrast, no significant changes in antral motility was evident comparing RX and RX + 5-HT injection into the DVC (Mean ± SEM; n= 3-5):

RX (4 pmoles)	RX	—	RX	RX	RX	RX
5-HT (pmole)	—	49	49	4.9	0.5	0.005
30 Min Mot. index (% basal)	270 ±70	139 ± 34	387 ± 98	320 ± 92	298 ± 63	256 ± 19

Systemic fluoxetine pretreatment did not alter RX (4pmol)-stimulated gastric motility after DVC injection. The results suggest that TRH analogue-stimulated GAO but not antral motility is enhanced by enhanced 5-HT levels in the DVC. Supported by DK 47432.

640.7

THE EFFECTS OF BOMBESIN ON THE VAGAL AFFERENT TRANSMISSION IN THE NUCLEUS TRACTUS SOLITARIUS (NTS) OF ANESTHETIZED RATS. H.J. Park[1], M.Y. Park[1], M.S. Kim[1], E.H. Lee[1], Y.L. Lee[1], H.C. Shin[1], S.C. Lee[2]* Dept. of Physiology[1], College of Med., Hallym Univ., Chunchon, Korea & Dept. of Physiology[2], College of Med., Ewha Womans Univ., Seoul, Korea

A dense plexus of bombesin immunoreactive terminals has been found in the NTS (Ladenheim *et al.*, 1992). Intracisternal injections of bombesin have ulcergenic effects in the stomach (Tache, 1982) and bombesin microinjected into the dorsal vagal complex inhibits TRH-stimulated gastric contractility in rats (Heymann-Monnikes *et al.*, 1990). These studies suggest that bombesin may play important roles in the control of visceral functions. The present study was carried out to test the effects of intracisternal administration of bombesin (0.001, 0.01, and 0.1 μg) on the NTS neuronal activities evoked by the electrical stimulation of the vagus nerve of anesthetized rats. Quantitative determination of the effects of bombesin on the afferent sensory transmission was made by generating post-stimulus time histograms. Low doses (0.001 μg) of bombesin did not change the afferent transmission during the 40 min post-injection period. However, medium doses (0.01 μg) of bombesin significantly ($P < 0.01$) inhibited the afferent transmission in the 16 to 35 min post-drug period (-23.9 ± 7.8%). High doses (0.1 μg) of bombesin exerted either significant inhibitory (-38.9 ± 13.7%, $P < 0.01$) or facilitatory (+27.1 ± 7.5%, $P < 0.05$) effects on the responsiveness of NTS neurons. These results indicate that endogenous bombesin-like peptide present in the NTS may participate in the processing of visceral afferent information (supported by KME 93' Basic Medical Research Fund granted to Dr. H.J. Park).

640.8

CENTRAL APPLICATIONS OF PEPTIDE YY (PYY) ALTERS GASTRIC FUNCTIONS VIA VAGAL PATHWAYS C.H.Chen, R.C.Rogers*, R.L.Stephens Jr. Dept. Physiol. Ohio State U. Columbus, OH 43210

A postprandially released ileal hormone, peptide YY (**PYY**), can inhibit proximal gut acvtivities and has been associated with the "ileal brake" phenomenon. In addition, circulating PYY has been shown to enter the dorsal vagal complex (**DVC**) region in the medulla [Hernandez, et al., AJP, 1994], which contains the "vago-vagal reflex" control circuits. Therefore, we predicted that central injection of PYY into the brainstem will alter gastric functions via vagal pathways. In the present study, two different parameters of gastric activity, antral motility and emptying, were monitored following central injection of PYY. 1) The antral motility data demonstrated that unilateral injection of PYY (20.0 and 2.0 fmol in 20 nl) directly into the DVC produced significant suppression of the thyrotropin-releasing-hormone (**TRH**) stimulated increase of the antral motility in urethane-anesthetized rats. This dose-dependent inhibitory effect was completely eliminated with an ipsilateral cervical vagotomy. 2) The effect of an intracisternal (**ic**) injection of PYY on the rate of gastric emptying was investigated in awake rats. The result showed that a dose-dependent inhibition of gastric emptying was observed following the ic injection of PYY (400, 40, and 0.04 fmol in 10 μl). Subdiaphragmatic vagotomy eliminated the PYY inhibitory effect on gastric emptying. These studies confirmed that PYY is able to inhibit gastric functions, such as motility and emptying, by acting via the DVC. These data support the hypothesis that PYY elicits the "ileal brake" effect on the stomach by altering the activity of vago-vagal reflex circuits. However, the specifics of the neuronal responses by components of the DVC to PYY application will require both in vivo and in vitro electrophysiological methods.
#These work were supported by NIH Grant 30803 to RCR and OH 47432 to RLSJ.

640.9

THE MECHANISMS OF ANTIULCER ACTION OF PRORANOLOL IN RATS. S. K. Kaan and C. H. Cho. Dept. of Pharmacology, The University of Hong Kong, Hong Kong. (SPON: The Hong Kong Society of Neurosciences).

Although propranolol, a non-selective beta-adrenergic antagonist, has been shown to have an antiulcer action, the mechanisms for this action are still unclear. The aim of this study was to investigate these mechanisms in three ulcer models, i.e. 60% ethanol, 30 mg/kg indomethacin and cold-restraint stress. The relationship between gastric mucosal blood flow (GMBF) and systemic blood pressure was also studied. Male Sprague-Dawley rats were used in the whole experiment. Pretreatment with propranolol (5 or 10 mg/kg) given either intraperitoneally or orally dose-dependently prevented gastric mucosal damage in these three ulcer models. The drug decreased GMBF which was probably due to its depression action on systemic blood pressure. Propranolol preserved the adherent and intramucosal mucus in all three ulcer models and increased the mucosal potential difference. In addition, the membrane stabilizing activity of propranolol may contribute to the elevation of potential difference. These findings indicate that propranolol strengthens the mucosal barrier and tightens the gap junction by the mechanisms of preservation of mucosal mucus and stabilization on mucosal membrane in the stomach. The action on GMBF does not responsible for the antiulcer action of the beta blocker.

640.10

RELATIONSHIPS OF IGLES TO ENTETRIC GLIA AND NEURONS IN THE RAT ESOPHAGUS: FURTHER INDICATIONS OF A MECHANOSENSOR-LOCAL EFFECTOR ROLE. W.L. Neuhuber*1, M. Kressel1, M. Dütsch1, J. Wörl1, and H.R. Berthoud2, 1 Anatomy Inst., Univ. Erlangen-Nürnberg, D-91054 Erlangen, and 2Pennington Biomed. Res. Ctr, Baton Rouge, LA 70808

Intraganglionic laminar endings (IGLEs) are prominent structures of vagal afferent origin with unknown function, distributed to enteric ganglia particularly in the esophagus and stomach (Berthoud&Neuhuber 1994). Combining anterograde tracing, immunocytochemistry, confocal laser scanning and electron microscopy, we attempted to gain more insight into structural details of IGLEs indicative of a possible function. Rat nodose ganglia were injected with WGA-HRP or DiI to anterogradely label IGLEs in the esophagus. Tissues were processed for electron microscopy (WGA-HRP) and immunocytochemistry for calretinin (DiI). Esophagi from untreated rats or from animals subjected to unilateral cervical vagotomy were processed for immunocytochemistry for calretinin or calbindin alone, or in combination with S-100 or vinculin. Results indicate that: 1) IGLEs are the only significant vagal afferent terminal structures in the striated muscle coat of the esophagus; 2) they are located mainly on the surface of myenteric ganglia immediately beneath the basal lamina. They contain numerous mitochondria, small clear vesicles and a fine filamentous "receptor matrix"; 3) IGLEs interdigitate with fine glial processes co-staining for both S-100 and vinculin which probably provide a three-dimensional framework connected to the perigangliona extracellular matrix; 4) IGLEs co-stain for both calretinin and calbindin, two markers which are typically found in rapidly adapting mechanosensors (Duc et al. 1993); 5) they form synaptic contacts with enteric neurons. Taken together, these data may indicate a mechanosensor function of IGLEs which may, in addition, synaptically influence enteric neurons as previously suggested (Neuhuber 1987). (Supported by SNF 3.555-86 and DFG/SFB 353/B9)

641.1

HEPATIC VAGAL AFFERENT INNERVATION OF THE GI TRACT.

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Metabolic and feeding functions have been correlated with the hepatic branch of the vagus and attributed to the liver, in part for lack of information about the branch's non-hepatic projection fields. Although hepatic motor projections to the GI tract have been partially mapped (AJP, 260, R200-7, 1991), neither the nerve's afferent projections to, nor terminal specialization's in, the GI tract have been described. To survey these afferents, male SD rats (n=11) were given partial subdiaphragmatic vagotomies sparing only the hepatic branch and then injected i.p. with Fluoro-gold (1mg). Each rat received an injection of 3 µl WGA-HRP in the left nodose ganglion 3 days before sacrifice. All animals were perfused 2 weeks after vagotomy. The medulla was sectioned (56 µm) and prepared to verify the vagotomy (Fluoro-gold: 3/4 of the sections) and nodose injection (HRP: 1/4 of the sections). The nodose ganglia, entire stomach, first 8 cm of duodenum, and cecum were prepared as wholemounts and processed with TMB. Different terminal types were counted with a sampling grid. Hepatic innervation of the ventral stomach consisted of one or two bundles entering at the lesser curvature and wandering to the fundus where they branched into terminal fields. The only fibers on the dorsal stomach were distal branches and terminals that wrapped around the greater curvature of the fundus from the ventral side. The hepatic branch supplied the fundus with both intramuscular arrays (IMAs-- which have been associated with tension reception) and intraganglionic laminar endings (IGLEs). Afferents were also supplied to the distal antrum and the pylorus (IMAs). Duodenal innervation was, overall, denser in the first 2 to 4 cm and dwindled progressively along the small intestine. Cecal afferents were sparse, consisting of a few fibers in and near the ileocecal junction. Duodenal and cecal afferent endings were predominately IGLEs. These results indicate that the hepatic branch carries information from the fundus, antrum, pylorus, duodenum, and cecum. Further, the different types of terminals it supplies suggest that the branch mediates a multiplicity of GI sensory functions. NIH DK27627 AND NIMH MH01023.

641.2

ACTIONS OF CHOLECYSTOKININ IN GANGLIA OF THE GUINEA PIG SPHINCTER OF ODDI.

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Following meals, cholecystokinin (CCK) acts in the sphincter of Oddi (SO), as well as in the gallbladder, to promote the flow of bile into the lumen of the intestine. Motility studies indicate that CCK's action in the SO is neurally mediated. SO ganglia are comprised of three types of neurons based on electrical properties [Wells and Mawe; Am. J. Physiol. 265: G258-H269, '93], and they contain distinct groups of neurons that express excitatory or inhibitory neuroactive compounds [Wells et al., J. Comp. Neurol. 352:106-116, '95]. To assess CCK's action in SO ganglia, intracellular recordings were made from 85 neurons in intact SO preparations, and CCK was applied by pressure ejection (0.1 mM, 20 PSI, 0.01 - 3.0 sec) or superfusion (1 - 100 nM). CCK had a direct excitatory effect on tonic and phasic cells in SO ganglia. In tonic cells (n= 23) CCK typically caused a prolonged depolarization that was accompanied by the generation of action potentials at high frequency during most of the depolarization. In phasic cells (n= 53/55), CCK caused a brief and/or prolonged depolarizations that were associated with increased excitability. In AH cells, CCK sometimes caused a slight depolarization(n= 3/7), and it caused an increase the amplitude and duration of the prolonged afterhyperpolarization. In summary, CCK has a direct, excitatory effect on the majority of SO neurons. Further studies will be required to determine whether CCK's ganglionic action results in an increased output from inhibitory neurons to relax the SO, and/or increased output from excitatory neurons to pump bile through the SO. Supported by DK 45410 & NS26995.

641.3

SYNAPTIC TRANSMISSION IN RABBIT PANCREATIC GANGLIA.

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Pancreatic ganglia are innervated by vagal, sympathetic, myenteric, and primary afferent nerves and provide the postganglionic parasympathetic innervation to pancreatic endocrine and exocrine cells as well as the pancreatic duct. Presently, little is known of the synaptic transmitters and resulting potentials which regulate the firing of pancreatic neurons and thus parasympathetic output to the secretory cells. To study this question portions of rabbit pancreas, with or without the duodenum attached were removed and intracellular recordings were made from pancreatic neurons in single or interconnected ganglia. Pancreatic neurons had an RMP of 51 ± 1mV (mean ± S.E.) and exhibited a phasic firing pattern. Single stimuli of attached nerve fibers evoked nicotinic fast EPSPs and action potentials while repetitive stimulation also revealed slow cholinergic and non-cholinergic EPSPs. Low frequency (0.5 Hz) nerve stimulation resulted in variable synaptic responses with frequent failures. Atropine (2µM) increased the mean synaptic response while reducing failures. Exogenous muscarine (10µM) decreased the mean synaptic response and increased failure frequency. At higher stimulus frequencies (5-10 Hz) facilitation of synaptic transmission occurred resulting in frequent action potentials with few failures even in the absence of any slow EPSPs. Application of norepinephrine (NE) or the α_1 agonist phenylephrine (5-10 µM) depolarized (8 ± 1mV) 8/19 neurons and facilitated synaptic transmission while 7/15 neurons responded to NE with hyperpolarizations (8 ± 1 mV) and synaptic inhibition. Adrenergic inhibition of synaptic transmission predominated in ganglia from the head/neck region of the pancreas while adrenergic facilitation was only observed in ganglia isolated from the body/tail region. Thus, a variety of synaptic mechanisms mediated by multiple neurotransmitters interact to determine the output of pancreatic neurons to the endocrine and exocrine pancreas. Supported by NIH

641.4

GLUTAMATE AND N-METHYL-D-ASPARTATE RECEPTORS IN THE PANCREAS: LOCALIZATION AND FUNCTION.

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Prior studies have suggested that N-methyl-D-aspartate receptors (NMDARs) are present in the pancreas. The present study was carried out to (i) locate NMDARs in this organ, (ii) determine if glutaminergic neurons are present in pancreatic ganglia; and (iii) determine whether visceral NMDARs can mediate long term potentiation (LTP). Glutamate (Glu) was detected immunocytochemically in pancreatic neurons in the guinea pig and rat. All of the neurons that were Glu-immunoreactive (ir) were co-stained with antibodies to ChAT. Glu-ir nerve fibers were found traveling along blood vessels and in the vicinity of acini. The majority of neurons in pancreatic ganglia expressed NMDARs. In addition, NMDARs were expressed by insulin-immunoreactive islet cells. NMDA depolarized pancreatic neurons and induced them to spike repetitively; this effect was blocked by the selective NMDA receptor antagonist, D-AP5 (50 µM) and Mg^{2+} (1 mM). Pancreatic neurons that responded to NMDA were surrounded by Glu-ir terminals. High frequency stimulation with trains of pulses (ten at 100Hz, 500 msec), delivered to intrapancreatic ganglionic connectives, potentiated the amplitude of fEPSPs to 138% of control. This potentiation lasted for 50 min and was blocked by the noncompetitive NMDA receptor antagonist, MK801. These observations are consistent with the idea that Glu and NMDARs participate in fast neurotransmission in pancreatic ganglia. The data are also consistent with the possibility that the synaptic release of Glu can stimulate NMDARs to induce LTP. Supported by grants NS01582 and the American Diabetes Association.

641.5

INHIBITION OF JEJUNAL ALANINE ABSORPTION BY VASOACTIVE INTESTINAL PEPTIDE: ROLE OF CAPSAICIN SENSITIVE PRIMARY AFFERENTS.

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The purpose of this study is to assess the role of capsaicin sensitive primary afferents (CSPA) in the inhibition of rat jejunal alanine absorption by vasoactive intestinal peptide (VIP). Continuous intravenous infusion of VIP (11.2 ng/kg-min) reduced alanine absorption by 60% in control rats and 24% in rats neonatally treated with capsaicin ($p<0.05$). In vitro, VIP decreased alanine uptake by jejunal strips in a dose dependent manner. In the presence of 20 nM VIP, alanine uptake by full thickness jejunal strips was reduced by 53% in control rats and by 19% in rats treated neonatally with capsaicin ($p<0.05$). On the other hand, the effect of VIP on alanine uptake by mucosal scrapings was similar in both groups of rats. In rats whose jejunae were pretreated with benzalkonium chloride (BAC), VIP's inhibitory effect on alanine uptake was markedly reduced. Kinetic analysis of jejunal alanine uptake in the presence and absence of VIP revealed that it reduced Na dependent alanine transport by decreasing the affinity of its transporter. We conclude that VIP's inhibition of alanine uptake is neurally mediated and involves a change in the affinity for alanine uptake.

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641.6

LOCALIZATION OF CALCIUM RECEPTOR EXPRESSION IN CELLS OF THE RAT MYENTERIC PLEXUS.

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We have previously mapped the expression pattern of calcium receptor (CaR) mRNA in cells in specific regions of the CNS and have postulated that the ability to sense changes in extracellular calcium may play an important functional role in a subset of CNS neurons. Systemic hyper- and hypo-calcemia are associated with disorders of intestinal motility and neurons in the myenteric plexus play a critical role in the regulation of gastrointestinal (GI) peristalsis. We have localized expression of CaR mRNA, using *in situ* hybridization, and CaR protein, using immunocytochemistry, in cells of the myenteric plexus of the rat GI tract. Stomach, duodenum, jejunum, ileum, proximal colon, and distal colon were assayed for rat CaR by PCR and CaR mRNA was detected in all of these regions. Sections from these same regions were hybridized with a ^{33}P-labeled riboprobe complementary to the rat CaR (antisense probe) or a control (sense strand) riboprobe. A subset of cells with cell bodies located in the myenteric plexus showed hybridization with the antisense CaR riboprobe; sense strand controls showed no hybridization. Adjacent tissue sections were stained with an antibody generated to a portion of the amino terminal region of the CaR and staining of a subset of cells in the myenteric plexus as well as processes that extended beyond the plexus was observed. This pattern of expression was present throughout the GI tract from stomach to distal colon. These observations suggests that CaR expressing cells in the myenteric plexus may play a role in the regulation of GI peristalsis. In addition, the presence of these cells throughout the length of the GI tract suggests that the CaR may be involved in the coordination of motility between different regions of the intestine.

641.7

IMMUNOHISTOCHEMICAL LOCALIZATION OF GLUTAMATE AND N-METHYL-D-ASPARTATE RECEPTORS IN THE ENTERIC NERVOUS SYSTEM. M.-T. Liu, M. Howard*, M.D. Gershon, and A. Kirchgessner. Dept. of Anat.& Cell Biol., Columbia Univ., NY, NY 10032.

Prior studies have suggested that N-methyl-D-aspartate receptors (NMDARs) are present in the gut. The present study was carried out to (i) locate NMDARs in the gut, and (ii) determine if glutamate-immunoreactive (Glu-ir) neurons are present in the enteric nervous system (ENS). Glutamate (Glu) immunoreactivity was detected in neurons in the submucosal and myenteric plexuses in all regions of the rat and guinea pig bowel. Glu immunoreactivity was also observed in paravascular nerves and nerve fibers in the enteric plexuses, longitudinal muscle and mucosa. In the guinea pig submucosal plexus, ~ 27 % of neurons were Glu-ir. All of the submucosal neurons that were Glu-ir were co-stained with antibodies to choline acetyltransferase (ChAT), substance P, and calbindin, which are putative primary afferent neurons. In the guinea pig myenteric plexus, ~ 5 % of neurons were Glu-ir, all of these contained ChAT, and those with a Dogiel type II morphology were calbindin-immunoreactive. In the rat bowel, ~ 53 % of submucosal and ~ 15 % of myenteric neurons were Glu-ir. In both plexuses, all Glu-ir neurons contained ChAT, and a subset were co-stained with antibodies to VIP, calbindin and calretinin. The majority of neurons in the ENS expressed NMDARs and, in a subset, there was coincident expression of NMDARs with Glu. These observations are consistent with the idea that enteric Glu and NMDARs play a role in the function of the ENS. The data are also consistent with the possibility that Glu may be a marker of submucosal intrinsic primary afferent neurons. Supported by NIH grants NS01582, NS12969 and the American Diabetes Association.

641.8

5′ UNTRANSLATED PREPROTACHYKININ DNA DIRECTS TRANSGENE EXPRESSION IN ENTERIC NERVES OF MOUSE SMALL INTESTINE. A.M.Yunker*, R.G. Lorenz, C.B. Latham, and K.A. Roth. Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110.

Products of the preprotachykinin (PPT) gene, such as substance P (SP) and other related peptides, are localized in subpopulations of neurons throughout the peripheral nervous system (PNS). Although sensory and autonomic nerves utilize tachykinins as neurotransmitters, the molecular signals that control cell-specific tachykinin expression are unknown. Therefore, transgenic mice containing either 5.5 kb of 5′ flanking region of PPT gene fused to human growth hormone (hGH) gene or 9.5 kb of 5′ PPT sequence fused to a non-bioactive mutant hGH reporter gene were generated to allow characterization of the DNA sequences necessary for cell-specific tachykinin expression. Seven lines of mice were established, and the pattern of transgene expression was examined in whole-mount preparations of ileum using immunohistochemical methods. Colocalization of SP- and hGH-like-immunoreactivity (-ir) was found in transgenic mice, but not non-transgenic littermates, from 2/2 lines containing the 5.5 kb construct and 4/5 lines containing the 9.5 kb construct, although disparate hGH- and SP-ir was observed. SP- and hGH-ir was localized in varicose and non-varicose nerves found within myenteric ganglia and interganglionic nerve bundles, and in nerves of the circular muscle plexus in 5/6 transgenic lines. Interestingly, hGH-ir was found only in occasional varicose nerves in myenteric and submucosal ganglia in 1/4 lines containing the 9.5 kb construct. Colocalization of SP- and hGH-ir was also found in submucosal preparations from animals containing either 5.5 or 9.5 kb construct, as staining was observed in paravascular nerves associated with submucosal arterioles, occasional submucosal neurons, and interganglionic nerves of the submucosal plexus. These data suggest that the 5′ flanking region of the PPT gene contains sufficient information to permit cell-specific tachykinin expression in the PNS.

641.9

CHEMICAL CODING OF NEURONS IN THE SMALL AND LARGE INTESTINE OF THE MOUSE. H.M. Young*, Q. Sang and J.B. Furness. Department of Anatomy and Cell Biology, University of Melbourne, Parkville, 3052, Victoria, Australia

The chemical coding of neurons in the small and large intestine of the mouse has been examined using immunohistochemistry. In both the small and large intestines, the largest classes of nerve cell bodies in the myenteric plexus displayed the following combinations of markers: NOS+/VIP+/NPY+, NOS+/VIP+/NPY-, NOS-/VIP-/NPY+, calretinin+/SP+, calretinin+/SP- and calretinin-/SP+. There was almost no overlap between NOS/VIP and SP, NOS/VIP and calretinin, NPY and calretinin or between NPY and SP. GABA and calbindin were also found in small populations of neurons. The calretinin-/SP+, calretinin+/SP-, NOS+/VIP+/NPY- and NOS-/VIP-/NPY+ were found to be interneurons, whereas circular muscle motor neurons had the coding NOS/VIP/±NPY or SP/±calretinin. By analogy with other species, it is likely that the NOS/VIP neurons are inhibitory and the SP neurons are excitatory motor neurons. Using myotomy and myectomy operations, the NOS/VIP neurons in both the small and large intestines were found to project anally, whereas the calretinin neurons appear to project locally.

641.10

TRANSNEURONAL LABELING OF NEURONS IN THE ADULT RAT CENTRAL NERVOUS SYSTEM (CNS) AFTER INJECTION OF PSEUDORABIES VIRUS (PRV) INTO THE DISTAL COLON. W. C. de Groat, M. Brisson, S.L. Erdman, G. Matsumoto*, J.R. Roppolo, J.P. Card and M.A. Vizzard. Univ. of Pittsburgh, Departments of Pharmacology and Neuroscience, Pittsburgh, PA 15261.

PRV tracing was used to identify sites in the CNS involved in the neural control of colonic function. PRV ($7X10^8$ pfu/ml; 1µl at three sites, 1-1.5" from anus) was injected unilaterally into the wall of the distal colon and the distribution of PRV-infected (PRV-I) neurons was examined (72,84,96 hr, n=2 at each time). In the L3-S1 spinal cord (S.C.), the majority of PRV-I cells were present in the S1 S.C. in the: 1) sacral parasympathetic nucleus (SPN; 72-96 hr), 2) dorsal commissure (DCM; 72-96 hr) and 3) dorsal horn (DH; 84-96 hr). The number of PRV-I cells in the S1 SPN was greater than that in L6 by a minimum ratio of 5:1 at 72 hrs. In the T13-L2 S.C., PRV-I cells were present in: 1) the intermediolateral cell column (72-96 hr), 2) DCM (72-96 hr) and 3) DH (84-96 hr). PRV-I DRG cells were present in the L1, L2, L6 and S1 DRG; however, most PRV-I cells were in L6-S1. Few PRV-I cells were present in the L5 DRG. S.C. sections (L1,L2,L6,S1) were also processed for NADPH-diaphorase activity (N-d; a presumed indicator of neuronal nitric oxide synthase). N-d positive (+) colon preganglionic neurons (PGNs) make up a much larger % (25.4%, L1) of N-d+, sympathetic PGNs compared to Nd+, parasympathetic PGNs (5.6%, S1). N-d fibers were present along the lateral edge of the L6-S1 DH extending into the SPN. N-d varicosities surrounded N-d negative, PRV-I cells in the dorsal region of the SPN. PRV labeling at 72-96 hr was also observed in a variety of supraspinal sites: DMV, NTS, inferior olive, A5, C1, hypothalamus, PAG, nucleus subcoeruleus, A7, Red nucleus (N.), N. ambiguus, raphe, central gray, Edinger Westphal N. and cortex and most notably the pontine micturition center (PMC); a region conventionally viewed as controlling bladder and urethral function. In conclusion, these results suggest that the PMC may have broader functions beyond those related to the control of the urinary tract and that the parasympathetic component (L6-S1) of colonic spinal neurons has a segmental distribution distinct from that of bladder or urethra.

641.11

LUMBAR SYMPATHETIC NERVES REGULATE NITRIC OXIDE-DEPENDENT NON-CHOLINERGIC CONTRACTIONS IN CAT COLON. K. Venkova and J. Krier*, Dept. Physiol., Michigan State Univ., East Lansing, MI 48823

The effects of lumbar sympathetic nerves (lumbar colonic nerves, LCN) on non-cholinergic contractile responses evoked by electrical stimulation of the pelvic nerve (PN) were studied "*in vitro*" by isometric force measurements of colon longitudinal and circular muscle in the presence of atropine (0.5µM). The preparation consisted of a mucosa-free smooth muscle sheet (2.5 x 4.5 cm) of cat mid-distal colon with attached LCN and ipsilateral pelvic plexus ganglia and PN. The smooth muscle preparation was pinned flat to the bottom of an organ bath (85 ml). Two muscle strips (2 mm wide and 3.5-4 mm long) were cut parallel to the longitudinal and the circular layer, respectively. The ends of each muscle strip remained attached to the smooth muscle sheet. Force transducers were attached to the middle of each muscle strip for 'bridge-like' recordings of mechanical activity from the longitudinal and circular muscle layer. Bipolar platinum electrodes were placed on the PN and the LCN. Electrical stimulation (2-5 Hz for 30 s) of the PN caused inhibition of spontaneous contractions followed by a sustained contraction of longitudinal and circular muscle after the period of stimulation. In both muscle layers, the non-cholinergic contractions were blocked by hexamethonium (50 µM) and reduced by 80 % in the presence of a nitric oxide synthase inhibitor, N-Nitro-L-Arginine (100 µM). Electrical stimulation of LCN (0.5-20 Hz) caused a frequency-dependent reduction of the nitric oxide-dependent PN-evoked non-cholinergic contractions. The inhibitory effect of LCN stimulation was abolished by guanethidine (50 µM). In summary, the data show that nitric oxide-dependent non-cholinergic contractions of longitudinal and circular muscle of cat colon are regulated by adrenergic fibers in LCN. (NIH-DK-29920)

641.12

BASAL NITRIC OXIDE INHIBITION ENHANCES NEURALLY-EVOKED CHLORIDE SECRETION IN GUINEA PIG DISTAL COLON. R.A. Reddix, A.J. DiMaggio, Jr., P.D. Feldman*. Dept of Pharmacology, LSU Medical Center, New Orleans, LA. 70112.

The aim of this study was to test the hypothesis that basal release of nitric oxide **(NO)** modulates neurally-evoked ion secretion in guinea pig distal colon. Submucosa/mucosa preparations were mounted in Ussing flux chambers. Changes in the short-circuit current (Isc) served as an index of active, electrogenic ion transport. Electrical field stimulation (**EFS**; 10Hz, 15 V, 0.5 msec) for 90 sec evoked a biphasic increase in Isc. Pretreatment with the NO chelator, hemoglobin (**Hb**; 15 mg/10 ml) significantly enhanced both peaks (pk1: 109.2 ± 2.8, pk2: 118.3 ± 4.8 %, n = 7-10, $p<0.05$) compared to vehicle (pk1: 93.1 ± 3.9 and pk2: 90 ± 3.3 %, n = 7-10, $p<0.05$). Secretion induced by carbachol (0.1µM), substance P (**SP**; 1 αM) and vasoactive intestinal polypeptide (**VIP**; 0.1 µM) was examined in the absence and presence of Hb. Sequestration of NO significantly enhanced secretion induced by VIP (Veh: 41.1 ± 9.3; Hb: 72 ± 13.5 %, n = 7, $p<0.05$) but had no effect on the carbachol and SP response. The results suggest that tonic NO release modulates neurally mediated increases in ion secretion in part by suppressing submucosal neural activity and postsynaptic VIP receptor activation.

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642.1

A NON-INVASIVE METHOD FOR QUANTIFYING PAIN-RELATED ACTIVITY IN THE HUMAN PRIMARY SOMATOSENSORY CORTEX

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We attempted to isolate scalp potentials generated by pain-related cortical activity by subtracting the somatosensory evoked potential (SEP) elicited by stimulation of the sural nerve at the pain threshold level from SEPs elicited at physiologically-defined noxious levels. This difference SEP included a mid-latency negative potential whose amplitude increased with increasing noxious stimulus intensity. The peak latency of the negative difference potential evoked by stimulation of the sural nerve at the ankle (106.5 ms) was longer than that evoked by stimulation of the nerve at a point 14.41 cm proximal to the ankle site (91.2 ms). (A similar latency difference was obtained from quantitative estimates of the onset of this potential.) These data demonstrate that the negative difference potential arises from activity in peripheral afferents with conduction velocities of about 9.4 m/s (i.e., A-delta afferents). A dipole source localization analysis of the negative difference potential demonstrated that it was best-fit (>95% of the variance) by a single source located in the primary somatosensory cortex (SI). Hence, this negative difference potential appears to reflect largely, if not exclusively, the response of SI neurons to noxious inputs. (Supported by N.I.H. NS28797.)

642.2

REGIONAL AND GLOBAL CEREBRAL BLOOD FLOW DURING PAIN PROCESSING BY THE HUMAN BRAIN.

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Mapping cerebral blood flow (CBF) using positron emission tomography (PET) now constitutes an important tool for the study of pain processing by the human brain. Despite the growing numbers of such investigations, pain-induced changes in both regional and global CBF have yet to be studied in a fully quantitative manner, a step critical for investigations of analgesic effects or graded responses during different pain states. We recruited pain-free volunteers to participate in a quantitative $H_2{}^{15}O$ PET study of CBF changes during both painful and non-painful stimulation of the upper right arm. Preliminary analyses (N=13) reveal that pain evoked by intradermal injection of 250µg capsaicin (CAP) produced robust global CBF **decreases**, from 48.7±2.3 ml/100g/min during rest to 37.0±2.1 ml/100g/min during CAP pain (ANOVA $p<0.0005$). These global blood flow changes exceeded those likely supported by the small (2.0mm Hg) but significant decrease in pCO_2 that occurred during CAP pain. CBF was normalized to global CBF to identify regional CBF differences between CAP, vibration (VIB) and resting states. CAP produced statistically reliable activation (Statistical Parametric Mapping, $p<0.001$) of brain areas likely to be involved in sensory and attentional processing, including the thalamus, anterior cingulate cortex, and the primary somatosensory cortex. Additionally, normalized CBF increases were detected in regions likely to be involved in the coordination of motor responses, including the putamen, superior colliculus, red nucleus, and cerebellum. Non-painful vibrotactile stimulation produced significant activation only in somatosensory fields adjacent to the posterior aspect of the lateral sulcus. These results confirm activation of sensory, attentional, and motor networks related to the pain experience and identify a previously undescribed global CBF decrease during severe pain. Furthermore, comparisons between CAP and VIB indicate that a robust painful stimulus may indeed produce greater activation of brain regions such as the thalamus and SI than a non-painful stimulus.

642.3

COINCIDENT OR DERMATOMAL THERMALLY INDUCED PAIN AFFECTS VIBROTACTILE SENSITIVITY.

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We have previously shown that heat-induced pain co-localized with vibratory stimuli can substantially increase detection thresholds (mean increase = 7.3 dB) in three of the four tactile channels (P, NP I, NPIII) (Apkarian, et al. *Somatosen. Motor Res., II*: 1994). This study was designed to extend these findings by measuring this pain-touch interaction in the fourth psychophysical channel (NP II), to determine site specificity of this effect, and to assess whether cold-induced pain acts similarly. Human detection thresholds (2AFC) were measured on the right thenar eminences of five normal observers across sessions in which pain site and thermal type was varied. First, thresholds were significantly elevated under heat-induced, co-localized pain with vibrations activating the NP II channel (mean increase = 4.8 dB). Second, heat-induced pain measurably increased thresholds when presented within the dermatome at different sites, non-coincident to the vibratory stimulus, though this effect was somewhat channel-specific: substantial increases were found for the NP I and NP III channels (2.4 dB and 2.9 dB, respectively) but not for P channel. Third, cold-induced pain elevated thresholds substantially, but to a lesser degree than heat-induced pain (mean increase = 3.6 dB). Pain was never found to induce changes in vibrotactile sensitivity when the painful stimuli were presented outside the dermatome, either ipsilaterally or contralaterally. Our current results suggest that, because dermatomal pain affects vibrotactile sensitivity, the locus of the pain-touch effect may be subcortical. Further, the effects across heat-induced and cold-induced pain suggest that the underlying fiber populations act differently in these pain-touch interactions.

642.4

INNERVATION TERRITORY OF SYMPATHETIC EFFERRENTS IN HUMAN SKIN.

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Microneurography techniques were employed to record from C-fibers in the peroneal nerve of healthy volunteers. The units were identified by their constant latency response to electrical stimulation of their terminals in the skin from intracutaneous needle electrodes at 4 s intervals. Responsiveness to physical stimuli (v. Frey filaments, feed-back controlled radiant heat) and sympathetic provocation tests was determined by slowed conduction velocity during the relative refractory period as previously described (Schmidt et al., J Neurosci. 15(1):333-341, 1995). Sympathetic efferent units were identified according to their characteristic reaction to maneuvers enhancing the sympathetic outflow. In a sample of 95 fibers nine units were found unresponsive to physical stimuli but responded to sympathetic provocation tests. Conduction velocities were lower (.75 ± .08m/s) than those of afferent units. Most of them were located in the toes and distal part of the foot dorsum. Spontaneous activity in these units was absent or that low that their innervation territories could be mapped by means of trains of transcutaneous electrical stimuli. Two units had 2 and 4 separate territories, resp. Mean innervated area was found to cover 133 mm^2 (24-255 mm^2). Innervation territories of sympathetic units were about of the same size throughout the different regions of the leg and foot including the toes. Two unit could be tentatively classified as vasoconstrictor and sudomotor unit resp. by cooling and warming the subject. We conclude that microneurographic techniques can be used to delineate cutaneous innervation territories of sympathetic efferents. A rough estimate of innervation density for sympathetic units was derived.

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642.5

Sex Differences in Pain Perception and PET Analysis of Cerebral Activation Patterns During Noxious Thermal Stimulation.

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Sixteen healthy subjects, matched for age and handedness received repetitive innocuous (40°C) and noxious (50°C) 5 sec thermal stimuli to the left forearm. Each subject rated the intensity of each stimulation series according to a magnitude estimation procedure in which 0 = no heat sensation, 7 = barely painful and 10 = barely tolerable. Sex differences in pain perception were tested for significance using the Students t-statistic. Females perceived the 50°C stimulus as being more painful than the males ($p = 0.003$). At the same time, positron emission tomographic (PET) scans of cerebral blood flow (CBF) were performed after an intravenous bolus injection of H215O (66 mCi). Four scans were acquired at each temperature. Mean CBF images were created for each experimental condition and oriented onto standardized stereotaxic coordinates. Subtraction images were created between conditions for each subject and averaged across subjects of the same sex. Statistical thresholds were established with corrections for multiple comparisons. Guided by a priori hypotheses the following structures were ssampled and showed significant percentage increases in CBF: contralateral thalamus, cingulate cortex, S2 and S1 cortex, insula, thalamus, medial dorsal midbrain and cerebellar vermis. Although females showed a trend for enhanced CBF in the contralateral frontal cortex, ipsilateral thalamus and cingulate cortex compared to the males, these differences were not statistically significant ($t = 6.34, 5.41$ and 4.02, respectively; threshold 7.6), perhaps due to the small sample size. These results indicate that males and females perceive pain differently and suggest these differences may be associated with differences in activation of the neural mechanisms mediating the sensory and, possibly, the affective components of acute noxious thermal pain.

642.6

TEMPERATURE LOCALIZATION WITHOUT TACTILE INFORMATION IN A DEAFFERENTED PATIENT.

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In SI cortex heat sensitive neurons have receptive fields (RFs) as small as a single digit (Kenshalo & Isensee, 1983). We have evaluated the contribution of thermal RF information to the localizaton of temperature in a patient with selective loss of large diameter primary afferent fibers.

A female patient (age 47) with A$\alpha\beta$ deafferentation was tested for spatial discrimination of thermal stimuli. Clinical tests showed a total loss of touch, vibration and kinesthesia senses for the body and parts of the head. A sural nerve biopsy revealed severe demyelination of large fibers. Five normal women (mean age 42) were also studied. Temperature localization was determined on the forearm and fingers (deafferented regions) and on the forehead (intact region). Thermal pulses (5s, 1-cm diameter thermode) were applied to 5 spots on the arm or forehead, separated by 2 cm, or on the glabrous finger tips. Cold (20°, 0°C), heat (40°, 49°C) and a thermally neutral stimulus (32°C) were tested.

Both the patient and controls localized 100% of the stimuli on the forehead. On the finger tips the controls localized all stimuli, but the patient performed below chance (<20% correct) for 32° and 40°C, at 45% with 20°, 60% at 0° and 67% at 49°C. On the forearm, neither the patient nor controls localized more than 75% of the stimuli. The ability of controls to localize the stimulus on the forearm was not affected by stimulus temperature (ANOVA, $p=0.2$), and at all temperatures except 32°C, the performances of the patient and controls did not signficantly differ (X^2, $p's>0.1$). At 32°C the patient localized only at chance level, and performed significantly worse than controls ($p<0.01$).

These data suggest that activity in thermal pathways can contribute to temperature localization. On the finger tips, tactile localization is superior to thermal localization, but on the forearm, thermal localization may be as accurate as tactile. Supported by the Canadian MRC and Québec FRSQ.

642.7

CEREBRAL ACTIVATION IN THE HUMAN BRAIN BY PAIN, TEMPERATURE AND AN ILLUSION OF PAIN. M.C. Bushnell*, A.D. Craig, E.M. Reiman, L.-S. Yun, and A. Evans. Univ. Montréal & Montreal Neurol. Inst., Montréal, Canada, and Good Samaritan Reg. Med. Ctr. & Barrow Neurol. Inst., Phoenix, AZ.

Pain and temperature sensations are known to have a strong anatomical association. A direct interaction between these senses is demonstrated by the thermal grill illusion, whereby a sensation of burning pain is produced when the skin is stimulated with interlaced bars of innocuous warm and cool. We studied the neural basis of this illusion in humans using functional brain imaging.

Positron emission tomography (PET) was used to measure regional cerebral blood flow (rCBF) following bolus injections of $H_2{}^{15}O$ in 11 normal volunteers during stimulation of the hand with neutral (34°C), innocuous cool (20°C), innocuous warm (40°C), painfully hot (47°C) and painfully cold (5°C) stimuli, as well as during the thermal grill illusion (interlaced bars of 20° & 40°C). Statistical brain maps were derived, and global searches of the brain and directed searches of thalamus and SI, SII, anterior cingulate and insular cortices were performed.

Compared to 34° stimulation, all stimuli, whether innocuous or painful, produced significant rCBF increases in contralateral insular cortex. Painful heat, painful cold and the thermal grill pain illusion produced additional rCBF increases in contralateral thalamus and anterior cingulate cortex. Painful heat activated SI cortex, and both painful and innocuous stimuli activated regions near SII.

These data indicate that whereas insular cortex is involved in both pain and temperature processing, anterior cingulate cortex is activated by pain. The finding that an illusion of pain caused by innocuous temperatures leads to activation patterns similar to true noxious stimulation suggests that integration of pain and temperature in thalamus and cortex underlies the illusion. Supported by the Flinn Foundation, Barrow Neurol. Foundation and the Canadian MRC.

642.8

FUNCTIONAL MAGNETIC RESONANCE IMAGING OF HUMAN SOMATOSENSORY AND CINGULATE CORTEX DURING PAIN AND PARAESTHESIA EVOKED BY MEDIAN NERVE STIMULATION. K.D. Davis*[1], M.L. Wood[2], A.P. Crawley[2] and D.J Mikulis[2]. [1]Div. of Neurosurgery and [2]Dept. of Radiol., U. of Toronto, The Toronto Hospital, Toronto, Ont., Canada M5T 2S8.

Positron emission tomography studies suggest that the primary somatosensory (SI) and cingulate (Cg) cortices play a role in the perception of noxious thermal stimuli. In this study, we investigated whether transcutaneous electrical nerve stimuli could be used with non-invasive functional magnetic resonance imaging (fMRI) techniques to study specific pain-related cortical activity in SI and Cg.

All data were collected from normal male and female subjects. Images were obtained with a 1.5T MRI scanner. Each protocol typically consisted of alternating sets of 6 images during a task and 6 images at rest for a total of 72 images. Conventional gradient echoes were used with the following parameters: TR=68ms, TE=40ms, ~6sec/image, 30x22cm FOV, 256x128 matrix, 4mm slice, 45° flip angle. Electrical stimuli were applied to the median nerve and the intensity of stimulation was adjusted in each subject to evoke either non-painful paraesthesia or pain in the distribution of the nerve. Activated regions were determined by statistical analysis of the mean intensity signal differences of each pixel during the task and controls.

Stimuli that evoked non-painful paresthesias activated discrete areas of S1 but did not activate Cg. Painful stimuli activated both SI and Cg. Cg activation was in the caudal portion of the anterior Cg, in or close to caudal area 24. In some subjects, increasing intensity of pain was associated with a larger region of activation within SI. In some cases, the mean intensity signal within an activated region correlated to the intensity of evoked pain. These data indicate that electrical stimuli and fMRI can be used to study pain-related cortical activity and confirm the involvment of SI and Cg in pain perception.

PAIN MODULATION: ANATOMY AND PHYSIOLOGY—HUMAN STUDIES

643.1

AFFERENT CONDITIONING PRODUCES DIFFERENT EFFECTS ON CLINICAL AND EXPERIMENTAL PAIN IN CHRONIC LOW BACK PAIN PATIENTS. G.Cheing and C.W.Y. Hui-Chan*. School of Physical & Occupational Therapy, McGill University, Montreal, Canada, H3G 1Y5.

The objective of this study was to determine whether *chronic* clinical pain, *acute* experimental pain, and the flexion reflex (FR), will be modified in a similar or different manner by transcutaneous electrical nerve stimulation (TENS) in chronic low back pain patients.

Thirty young subjects suffering from low back pain longer than six months were studied. They were matched with respect to gender and severity of pain, then randomly assigned to two groups, receiving either TENS or sham TENS to the lumbro-sacral region for 60 min. The FR was elicited by a maximally tolerable electrical stimulation applied to the subject's right sole, and recorded electromyographically from the biceps femoris and tibialis anterior. Subjective pain sensation of the low back pain (clinical pain) and the electrical stimulation (experimental pain) were measured by two separate visual analogue scales. ANOVA and Tukey tests were used to analyze the data obtained *before, during* and, up to 60 min *after* TENS or sham stimulation.

The pre-stimulation control value of subjective low back pain sensation was decreased to 62.9% in the TENS group ($p<0.01$), but not in the placebo group. In contrast, there was no significant decrease in both the experimental pain sensation and the FR *before, during* and *after* TENS or sham stimulation within each group, and between the two groups. These results show that chronic and acute pain were modified by TENS in a different manner. It is known that the neural pathways mediating the two types of pain are different. Our present findings suggest that there could also be different anti-nociceptive mechanisms for chronic and acute pain in chronic low back pain patients.

643.2

MORPHINE AND SKIN BLOOD FLOW (SBF) CHANGES IN NEWBORN INFANTS. T. N. K. Raju* T. Roohey A. N. Moustagiannis and K. M. McCulloch. Dept. Pediatrics, Univ. of Illinois, Chicago IL 60612.

We have shown that SBF increases with pain in newborn infants. Here we assessed morphine-induced SBF using a non-invasive, laser Doppler method 20–30 min before, 20–30 min during, and 35–45 min after placement of percutaneous central venous catheters (PCVC) in 18 infants (median birth weight 1100 g, age 5 days). Ten of these were given 0.05–0.1 mg/kg IV morphine just before PCVC placement and 8 were not. Changes in hear rate (HR) and SBF (ml/100g/min) are shown below.

Variable	Group	Baseline	During	After
SBF	Morphine.	22.5±9.5	22.6 ±7.7	19.3±7
	No morphine.	23.7±8	45.3±34†	21 ±5
HR (bpm)	Morphine	156±13	154 ±13	151±13
	No morphine	161 ±11	168 ±9 †	167 ±11†

(†P <0.05 vs baseline & morphine group.) Respiration and O_2 saturation changed minimally in both groups. Without morphine SBF increased in 7/9 (overall $\bar{x}$ change +97%, $P < 0.05$); with morphine SBF changes were minimal during PCVC placement. SBF changes returned to baseline during recovery in both groups. We conclude: 1) IV morphine prevented a substantial SBF increase from pain, perhaps reflecting an analgesic effect; 2) non-invasive SBF can serve as a measure of pain and analgesia.

643.3

EFFECT OF CAPSAICIN ON HUMAN JAW REFLEX EVOKED BY ELECTRICAL TOOTH PULP STIMULATION. [1]P. Kemppainen*, [1]A. Waltimo, [1]T. Waltimo, [1]M. Könönen and [2]A. Pertovaara. [1]Dept. of Prosthetic Dentistry and [2]Dept. of Physiology, Univ. of Helsinki, Finland.

The effect of selective activation of nociceptive primary afferent fibers by capsaicin on a masseteric reflex was studied in healthy human subjects. The inhibitory masseteric reflex was evoked by constant current (single pulses) stimulation of the upper incisor. The sensation of the tooth pulp stimulation was evaluated by visual analogue scale (VAS). The magnitude of the reflex response was determined by jaw force measurements and electromyographic recordings (EMGs) from the active masseter muscle. The inhibitory masseteric reflex could be induced already at nonpainful tooth pulp stimulation and the magnitude of the reflex was elevated with increasing current values. Capsaicin (1 %) applied topically to the skin of the cheek produced a burning spontaneous pain sensation. During capsaicin treatment the VAS ratings for the tooth pulp stimulation were significantly reduced, whereas no marked changes were found in the magnitudes of the jaw reflex responses. Thus, the influence of selective activation of nociceptive fibers of the facial skin by capsaicin on the tooth pulp-evoked sensory and reflex responses differs from the effect of capsaicin on a nociceptive withdrawal reflex of the limb.

643.4

EFFECT OF ACUPUNCTURE ON SYMPTOMATIC KNEE OSTEOARTHRITIS. R.H. Wong*, B.M. Berman, L. Lao, P. Langenberg, M. Esfahani, and M.C. Hochberg. Departments of Family Medicine, Epidemiology and Preventive Medicine, and Medicine, University of Maryland School of Medicine, Baltimore, MD 21201

Osteoarthritis is a common disease in which there is no specific or curative therapy. The aims of this study were to compare self-reported pain, stiffness and physical function for patients receiving acupuncture plus conventional therapy or conventional therapy alone in patients with osteoarthritis of the knee and to assess the possible adverse effects of acupuncture. Seventeen patients, mean age 70±8 years, with moderate to severe osteoarthritis of the knee (fulfilling the American College of Rheumatology (ACR) classification criteria) were enrolled and randomized into either a control (n=9) or acupuncture group (n=8). The control group received baseline anti-rheumatic drug therapy for eight weeks. The treatment group was treated twice a week for eight weeks with acupuncture. Outcome assessments of pain and physical function using both the Western Ontario and McMaster (WOMAC) and the Lequesne Osteoarthritis Indices, and a fifty-foot walk time were performed at baseline and four-week intervals with follow-up at weeks 12 and 24. The WOMAC Osteoarthritis Index indicated that the acupuncture group improved significantly at weeks 4 ($p\leq0.05$), 8 ($p\leq0.02$), and 12 ($p\leq0.01$) using Wilcoxon's signed rank test for paired data with two-tailed P values. The Lequesne Index also showed significant improvement at week 8 ($p\leq0.05$) for the acupuncture group. There was no significant difference found in the fifty-foot walk times, and no significant adverse effects reported after acupuncture treatment. For the control group, no significant differences were found in the outcome assessments. The results indicate that acupuncture is associated with significant improvement in pain and physical function in patients with osteoarthritis of the knee. Support provided by NIH RFA#1-R21-RR09327-01.

643.5

GABA$_B$ AGONIST, BACLOFEN, REDUCES CENTRAL PAIN WITHOUT IMPAIRING SOMATIC SENSATION.

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Intrathecal injection of 50μg baclofen suppressed central pain in post stroke patients (Taira 1994). Substantial pain relief (pain score 1-2/10) lasted for 12-24 hours in five of six patients. On the contrary, somatic sensation and motor control were not impaired by intrathecal baclofen. First, we investigated the discrepancy in effects of baclofen on pain and somatic sensation. Field potentials evoked by sciatic nerve stimulation were recorded in the dorsal horn of the spinal cord in anesthetized rats. Early components (within 2 ms latency) of field potentials evoked by low intensity stimulation were not affected by application of 15μg baclofen. The early components of field potentials recorded in this experiment may involve single or a few synapses. In contrast, the amplitudes of late components (80 ms latency) evoked by high stimulus intensity decreased to 55% of the control value. Next, using the H reflex, we demonstrated that 15 μg baclofen had no effect on H wave amplitude. The results suggest that baclofen, in the spinal cord, has little effect on single synaptic responses. This may account for the discrepant effects on pain, somatic and motor control.

643.6

INTERACTIONS BETWEEN LOW THRESHOLD MECHANORECEPTORS AND NOCICEPTORS DURING SECONDARY HYPERALGESIA.

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Hyperalgesic states are characterized by an increase in the pain evoked by nociceptor stimulation and by a central alteration in the processing of low-threshold mechanoreceptor input such that their stimulation evokes pain (allodynia). In this study we have examined if stimulation of low-threshold mechanoreceptors from an area of secondary hyperalgesia could evoke nociceptor activity expressed as local vasodilatations (axon reflexes).

Experiments were conducted on normal human volunteers. Cutaneous blood flow and temperature in two different points were measured with a Laser Doppler instrument. Hyperalgesia was induced by the application of mustard oil (25%) or by intradermal injections of capsaicin (25-50 μg) in the volar skin of one forearm. Stimulation of low threshold mechanoreceptors was achieved by brushing or stroking the skin. Pain ratings (VAS) and simultaneous recordings of blood flow and skin temperature were obtained.

Stimulation of low threshold mechanoreceptors in normal skin before the induction of secondary hyperalgesia or outside these zones once induced, did not evoke changes in skin blood flow. However gentle stimulation of the skin in areas of secondary hyperalgesia evoked painful sensations as well as localized increases in blood flow. These changes required continuous low intensity mechanical stimulation and outlasted the duration of the stimulus. Cooling the area of primary hyperalgesia reduced the vascular responses and the pain sensation evoked by gentle mechanical stimulation. These results show an interaction between low threshold mechanoreceptors and nociceptors appearing simultaneously with the development of the central changes of secondary hyperalgesia. Support: DGICYT APC-93-0102 and PB-93-0491.

643.7

CHANGES IN SKIN AND MUSCLE SENSITIVITY IN DYSMENORRHEIC VS NORMAL WOMEN AS A FUNCTION OF BODY SITE AND MONTHLY CYCLE.

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Changes were investigated in skin and muscle sensitivity of 7 dysmenorrheic vs 7 normal women as a function of body site inside and outside the metameric field of reproductive organs and monthly cycle. Pain thresholds to electrical stimulation were measured for *two abdominal areas* (inside reproductive field--symmetrical sites of the left and right rectus abdominis and overlying skin, 4 cm lateral to the navel) and for *two limb areas* (outside reproductive field--deltoid and quadriceps and overlying skin of one side). Measurement was repeated 4 times during a 28-day cycle; i.e., on days 2-6, during menstruation (phase a); 12-16, periovulatory (phase b); 17-22, luteal (phase c); 25-28, premenstrual (phase d).

Presence of dysmenorrhea: thresholds in dysmenorrheic women were normal in skin but lower than normal in muscle in every site with a significant difference for the abdomen in all phases and for the deltoid in phases a and b. Body site: in dysmenorrheic and normal women muscle thresholds were lower in the abdomen than in the limbs. Cyclic variations: in dysmenorrheic and normal women the highest threshold value was in the luteal phase (phase c) for both skin and muscle while the lowest occurred periovulatorily (phase b) for skin and perimenstrually (phases a or d) for muscle. This monthly trend was significant in muscle in every site for dysmenorrheic women but only in the abdomen for normal women.

Hyperalgesia is evident in muscle but not in skin both inside and outside the metameric fields of reproductive organs for dysmenorrheic women but only inside them for normal women. Sensitivity increases periovulatorily in skin and perimenstrually in muscle and decreases in the luteal phase for both tissues regardless of body site and dysmenorrhea status, but a significant monthly rhythm appears only in hyperalgesic areas. (KJB was supported by funds from NIH grant NS 11892; all other authors were supported by funds from CNR FATMA grant 94.00671.PF41).

PAIN MODULATION: ANATOMY AND PHYSIOLOGY—BRAINSTEM

644.1

EFFECTS OF UNILATERAL NUCLEUS TRACTUS SOLITARIUS LESIONS ON CENTRALLY-MEDIATED HYPERALGESIA.

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Endogenous hyperalgesia circuitry can be activated in response to a variety of aversive events. Specifically, illness-causing agents (such as emetics or pyrogenics) have been shown to produce internal aversive occurrences that result in the enhancement of pain sensitivity as measured by the tail-flick test (TF). Recent evidence has also shown that s.c. formalin (right hindpaw) activates a centrifugal pathway that results in hyperalgesia measurable using the TF test. While the neural circuitry responsible for these centrally mediated hyperalgesias has only recently come to be investigated, current evidence strongly suggests that illness-induced hyperalgesia originates in the viscera at vagal terminals, implicating that the ascending message may involve mediation in the hind brain at the nucleus tractus solitarius (NTS).

The present studies investigated the possibility that lesions (unilateral, electrolytic) of the NTS would disrupt centrally mediated hyperalgesias. After a recuperative period, pain responsivity was tested in lesioned and sham-lesion control animals following injection of lipopolysaccharides (LPS; intraperitoneal, IP; pyrogenic) lithium chloride (LiCl; IP, emetic), or formalin (right hindpaw; irritant). Preliminary results indicate that NTS lesions disrupt hyperalgesias produced by LPS and LiCl, but not formalin. Supported by NIH/NIDA grant 1R29DA09289-01 to EPW.

644.2

PROJECTION OF MET-ENKEPHALIN NEURONS TO NORADRENERGIC CELLS IN THE DORSOLATERAL PONTINE TEGMENTUM: A POSSIBLE ROLE IN MODULATING NOCICEPTION.

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The dorsolateral pontine tegmentum (DLPT) plays a major role in modulating nociception. Neurons in the ventromedial medulla (VMM) project to the DLPT and activate spinally-projecting neurons which modify nociceptive stimuli. We recently demonstrated that a population of these VMM neurons are immunoreactive for methionine-enkephalin (mENK) and can be retrogradely labeled from the DLPT. Met-Enk terminals were also found in apposition to tyrosine hydroxylase-immunoreactive (TH) cells in the DLPT, suggesting that mENK cells from the VMM innervate noradrenergic cells in the DLPT.

To verify these findings, iontophoretic injections of the anterograde tracer, Fluoro-Ruby (FR), were made into the VMM. Axons from cells in the VMM were double-labeled with both FR and mENK. These double-labeled axon terminals were found in apposition to TH cells in the DLPT. A number of double-labeled terminals were also found in other areas known to participate in antinociception, including the parabrachial region, the nucleus reticularis pontis oralis (RPOo) and the subcoeruleus region. These findings suggest that the VMM mENK neurons that innervate spinally-projecting noradrenergic cells in the DLPT are involved in modulating nociception. (Supported by USPHS grant DA03980, National Institute on Drug Abuse and NR07075-03, National Institute of Nursing Research).

644.3

SUBCUTANEOUS FORMALIN INDUCED ACTIVITY OF ON- AND OFF-CELLS OF THE ROSTRAL VENTROMEDIAL MEDULLA.

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The formalin (FOR) test is widely used to measure behavioral responses to a long-lasting nociceptive stimulus capable of eliciting a sequence of pain-related events characterized by an early (acute) phase and a late (tonic) phase, but the neural mechanisms involved have not been clearly identified electrophysiologically. This study evaluates the possible role of medullary on- and off-cells, which are putative nociception-modulating neurons that project to the spinal cord, during the time course of the effect of FOR.

The activity of medullary neurons was recorded in rats during application of noxious heat to the tail or noxious pinch to a paw. Thiopental anesthesia kept the animals free from signs of pain or discomfort while stable responses to noxious stimulation occured. On- and off-cells were characterized, since on-cells increase and off-cells stop their firing just before tail-flick or immediatly after noxious pinch. Thereafter FOR (100 µl, 10%) was subcutaneously injected in one of the hindpaws and the responses of the on - and off-cells were studied for 65 min.

FOR produced both early and late excitatory responses on the firing frequency (358.64 and 242.54% respectively) in all the on-cells tested, but only inhibition of activity (61.12%) during the early phase in the off-cells. During the late phase the off-cells showed only a reduction of 16.67% in their firing frequency. This suggests the existence of a new possible balance of activity between the two classes of neurons, probably induced by the tonic situation, in which the supressive effect of off-cells during the late phase probably is not sufficcient to cause inhibition of on-cells. Also, the interphase period, behavioraly defined as a zone of no pain, can be the result of the continuous firing observed during this interval in the off-cells.

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644.4

EFFECTS OF "DEEP MUSCLE PAIN" ON TRIGEMINAL MOTONEURONS AND INTERNEURONS DURING FICTIVE MASTICATION.

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Evaluation of data from clinical studies show that chronic muscle pain is not associated with tonic hyperactivity, as postulated by the vicious cycle theory. Instead, resting EMG activity appears normal. During function, agonist activity is less than normal, while antagonist activity tends to rise slightly. Experimental pain causes similar changes in muscle activity in man and animals. The specific aim of the present study was to evaluate how nociceptive muscle inputs act on the brainstem oro-facial motor circuitry during masticatory movements. Rabbits were anaesthetized with halothane, decerebrated at the precollicular level and paralyzed. Fictive mastication was evoked by repetitive stimulation of the cortico-bulbar tract and monitored in the trigeminal motoneuron subnuclei. Muscle pain was induced by injections (160µl) of hypertonic saline (5%) into the deep portion of the masseter muscle. Changes in the masticatory motor pattern and the firing properties of interneurons located in the oral subnucleus of the spinal trigeminal tract, were simultaneously analyzed before, during and after fictive mastication induced in the presence or absence of the noxious stimulations. Our results show that muscle nociception reduces the masticatory frequency and the frequency of discharge of the jaw motoneurons. Clear modifications of the firing frequency were also observed in 11 out of 13 interneurons that were rhythmically active during fictive mastication. An increased firing frequency was the main finding. Our results suggest that muscle nociceptive inputs adapt the movement pattern through actions on the rhythm and burst generating components of the brainstem central pattern generator for mastication.

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644.5

THE ROLE OF THE ROSTROVENTRAL MEDULLA (RVM) IN I.V. SEROTONIN PRODUCED INHIBITION OF THE TAIL FLICK (TF) REFLEX IN RATS.

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I.v. administration of serotonin inhibits the TF reflex through activation of vagal afferents. Previous studies with electrical stimulation of vagal afferents showed that vagal stimulation inhibits OFF cells and excites ON cells of the RVM at intensities that inhibit the TF reflex. The current study examined the possible role of the RVM in i.v. serotonin-induced antinociception.

Serotonin dose-dependently attenuated the TF reflex and produced the Bezold-Jarish reflex. Lidocaine microinjections into the RVM attenuated the inhibition of the TF reflex produced by 80 µg serotonin. Electrophysiological recordings in the RVM showed that serotonin predominately inhibits OFF cells and excites those ON cells with low background activity. Bilateral cervical vagotomy decreased the background activity of OFF cells and increased the background activity of ON cells and attenuated the serotonin-produced changes in neural activity. These findings are similar to the earlier findings with electrical stimulation of vagal afferents.

Supported by NIH grant NS31495

644.6

ANTINOCICEPTION AND IMMOBILITY PRODUCED BY MICROINJECTION OF LOW DOSES OF KAINIC ACID INTO THE RVM OF THE RAT.

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The pathway for modulation of nociception from the periaqueductal gray to the rostral ventromedial medulla (RVM) to the spinal cord has been well characterized. Antinociception mediated by this circuit appears to be part of a defensive reaction. For example, activation of the ventrolateral PAG produces both antinociception and immobility. Because the PAG projects to the RVM, the present study examined whether the RVM is part of the pathway mediating immobility.

Male Sprague-Dawley rats were chronically implanted with a guide cannula aimed at the RVM and injected with low doses of kainic acid (4, 20, & 40 pmol in 200 nL) or saline. Microinjection of kainic acid into the RVM produced a dose dependent inhibition of locomotion in the open field test (i.e. no effect with 4 pmol vs a maximal effect with 40 pmol) even though rats were capable of movement. Moreover, immobility in the open field was consistently associated with antinociception on the hot plate test.

The immobility and antinociception seen in the present study are similar to the effects produced by activating neurons in the ventrolateral PAG and suggest, along with anatomical and physiological data, that these behaviors are mediated via a ventrolateral PAG to RVM pathway. Presumably, the PAG and RVM are part of an integrated defense circuit coordinating responses to potentially life threatening situations.

Funded by a grant from the Washington State University Alcoholism and Drug Abuse Program.

644.7

DISTRIBUTION OF TYROSINE HYDROXYLASE IMMUNOREACTIVE (TH-IR) APPOSITIONS ONTO PHYSIOLOGICALLY IDENTIFIED NEURONS IN THE RAT ROSTRAL VENTROMEDIAL MEDULLA.

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TH-IR fibers and terminals are present throughout the rostral ventromedial medulla (RVM) and the activity of RVM neurons is influenced by noradrenergic inputs. There are two classes of putative pain modulating neurons in RVM: on-cells and off-cells, which respectively burst or pause prior to withdrawal reflexes. On-cells respond to iontophoretic application of noradrenaline (NA) and other alpha-adrenergic receptor agonists. To better understand the contribution of NA to the control of RVM neurons, we analyzed the anatomic distribution for catecholaminergic termination upon these two physiological cell classes in RVM.

On- and off-cells were identified by the change in their discharge rate in relation to nociceptive withdrawal reflexes and labeled by intracellular injection of neurobiotin in the lightly anesthetized rat. Sections containing labeled cells were visualized with a Texas red fluorophore conjugated to avidin and were subsequently processed for TH-IR using goat anti-rabbit IgG as a secondary antibody conjugated to a Bodipy fluorophore. The distributions of the two fluorophores were then mapped using a confocal laser scanning microscope (BIORED 600).

Seven RVM neurons (4 on, 3 off) were intracellularly labeled. Each on- and off-cell had a number of close TH-IR appositions. Appositions more commonly occurred on dendrites than on the somas of on-cells. There was a trend toward greater average density of TH-IR appositions onto on-cells.

These observations provide evidence that putative pain modulating neurons in RVM receive direct noradrenergic inputs.

(This work is supported by NIH grant NS 21445)

644.8

$GABA_A$ RECEPTOR SUBUNITS ON BULBOSPINAL NEURONS OF THE RAT.

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Microinjection of $GABA_A$ receptor ligands in the n. raphe magnus (NRM) or n. reticularis gigantocellularis pars α (NGCpα) modulates nociceptive sensitivity. It is not known whether these effects are mediated by spinally-projecting neurons of the NRM and NGCpα. This study examined whether neurons of the NRM and NGCpα that project to the spinal cord also possess $GABA_A$ receptors. Male S-D rats were perfused with 4% paraformaldehyde 10-14 days after injection of 2% Fluoro-Gold (FG) in the spinal cord. Transverse 25 µm sections were incubated in rabbit anti-serotonin (5HT) antiserum and either guinea pig anti-α_1 (1-16) subunit or guinea pig anti-α_3 (1-15) subunit antibodies. Other sections were incubated in goat anti-5HT antiserum and rabbit anti-α_1 (1-9) subunit antibodies. Primary antisera were visualized using CY3- or FITC-linked secondary antibodies. Neurons in the NRM and NGCPα were immunoreactive (IR) for the α_1 subunit, but none of these neurons were IR for 5HT. Of the α_1-IR neurons, a portion projected to the spinal cord as indicated by the presence of FG. NRM and NGCPα neurons were also IR for the α_3 subunit. Many, but not all of these neurons were also IR for 5HT. A portion of both populations of α_3-IR neurons contained FG. The differential distribution of α_1 and α_3 subunits, which confer different pharmacologic properties to $GABA_A$ receptors, suggests that NRM and NGCpα neurons that project to the spinal cord may be modulated by different types of the $GABA_A$ receptor. Supported by DE11423 and DA07255.

644.9

MORPHINE AND SOMATOSTATIN INTERACT TO REDUCE FOS-LI WITHIN TRIGEMINAL SUBNUCLEUS CAUDALIS AFTER CORNEAL STIMULATION IN THE RAT. D.A. Bereiter* and B.H. Tonnessen. Depts. of Neuroscience & Surgery, Brown Univ/RI Hospital, Providence, RI 02903

Corneal stimulation at noxious intensities produces Fos-like immunoreactivity (Fos-LI) in two spatially distinct transition regions of the spinal trigeminal nucleus (Vi/Vc and Vc/C1). Fos is the protein product of the immediate early gene c-*fos* and is a reliable marker for nociceptive-responsive neurons in the medullary dorsal horn. Morphine and somatostatin have been implicated as putative mediators of antinociceptive behavior among central trigeminal neurons. To assess the role of mu opioid and somatostatin receptor activation on Fos-LI, morphine (0.01-10 nmol) or octreotide (long-acting somatostatin analog, 0.1-1 nmol, icv) was given alone or in combination 20 min prior to corneal application of mustard oil to male rats anesthetized with pentobarbital. Mean arterial pressure (MAP) and heart rate (HR) were monitored in all groups. Neither morphine nor octreotide affected Fos-LI at the periobex Vi/Vc level, in ventrolateral medulla or NTS. In contrast, both morphine and octreotide caused a dose-related decrease ($P < 0.01$) in Fos-LI among laminae I-II neurons at the Vc/C1 transition level. Combined morphine (0.01 nmol) plus octreotide (1 nmol) caused a greater reduction in Fos-LI in laminae I-II at Vc/C1 than the additive effect of each drug alone. Morphine attenuated the MAP and HR responses to corneal stimulation in a dose-related manner, whereas octreotide had no consistent effect when given alone or in combination with the lowest dose of morphine. The magnitude of Fos-LI among laminae I-II Vc/C1 neurons was well-correlated with the MAP response to corneal stimulation under different doses of morphine ($r = 0.63$, $P < 0.01$), but not after octreotide. The results indicate that morphine and somatostatin interact centrally to reduce the expression of c-*fos* among corneal-responsive Vc/C1 neurons, but not Vi/Vc neurons. The neural substrate that mediates the apparent interaction between morphine and somatostatin and c-*fos* expression is separate from that which mediates the autonomic responses to corneal stimulation. Supported by NIH grant NS 26137.

PAIN MODULATION: PHARMACOLOGY—NMDA AND NO

645.1

REPETITIVE STIMULATION AND NALOXONE FOLLOWING OPIOID EXPOSURE INDUCE NMDA RECEPTOR-DEPENDENT LONG TERM POTENTIATION (LTP) IN ISOLATED RAT SPINAL CORD. J.-Q. Feng and J. J. Kendig*, Dept. of Anesthesia, Stanford Univ. Sch. of Medicine, Stanford, CA 94305

Recent evidence suggests that hyperalgesia induced by prolonged noxious stimulation and by precipitated withdrawal from opioids may have mechanisms in common. In isolated neonatal rat spinal cord, a prolonged (30 min or longer) significant increase in area of a nociceptive-related slow ventral root potential (sVRP) was induced by either tetanic (10 s^{-1}, 1 min) stimulation to a dorsal root (124% of control ± 22.4, mean ± SD, N=28) or by naloxone 200 nM following morphine 200 nM (142±17.5, N=6) or alfentanil 200 nM (148%±29.2, N=11). MK-801 20 nM had no effect on sVRP area alone but given before the tetanus or coadministered with the opioids blocked the increase in sVRP area induced by either tetanic stimulation or opioid. MK-801 20 nM shifted the morphine dose-response curve for sVRP depression significantly to the left. The results are consistent with a prolonged NMDA receptor-mediated excitatory effect exerted by repetitive stimulation and by μ opioid exposure. The phenomenon may represent the first step in a central sensitization that underlies both tolerance and neuropathic pain.

645.2

PHARMACOLOGICAL CHARACTERIZATION OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS IN SPINAL CORD OF NORMAL AND NEUROPATHIC RATS H. Wei*, L.B. Jakeman, and D.W. Bonhaus, Syntex Research, Palo Alto, CA 94304

Spinal cord NMDA receptors play a key role in nociception and in the sensitization of neurons following nociceptive input (wind up). Thus, NMDA receptor antagonists with selectivity for spinal cord receptors may be useful analgesics and may prevent the development of hyperalgesia or allodynia following nerve injury. We characterized the NMDA receptors present in spinal cords of rats with a painful neuropathy of the left hind foot (the "Bennett" model). Receptors were characterized in membrane homogenates and by receptor autoradiography using [^{3}H]MK 801 or [^{3}H]TCP. No difference in the density of NMDA receptors was detected between normal and neuropathic rats in the L4-L5 region of the spinal cord (fmol/mg protein equivalent; mean ± sem).

		Left dorsal horn	right dorsal horn
control	N=6	69.6 ± 6.6	64.8 ± 6.8
neuropathic	N=6	74.9 ± 6.9	71.9 ± 2.7

However, spinal cord NMDA receptors could be distinguished from those in brain cortex. Spermidine allosterically increased [^{3}H]TCP binding to NMDA receptors in brain cortex membranes (39±8%) but had no effect on [^{3}H]TCP binding to spinal cord membranes (2±1%). In this regard spinal cord NMDA receptors resembled those in the cerebellum (0±2%). These findings indicate that while there may be no change in the density of spinal cord NMDA receptors in neuropathic rats, spinal cord NMDA receptors can be distinguished from receptors in the cortex. Thus, it may be possible to develop NMDA receptor antagonists with selectivity for the spinal cord.

645.3

DIFFERENTIAL INVOLVEMENT OF EXCITATORY AMINO ACID RECEPTORS IN MEDIATING HYPERRESPONSIVENESS OF DORSAL HORN NEURONS IN NEUROPATHIC RATS. J.W. Leem*, E.S. Park, E.J. Choi, T.S. Nam and K.S. Paik. Dept. of Physiology, Yonsei Univ. Col. Med., SEOUL, 120-752, Korea.

The aim of this study is to investigate the roles of excitatory amino acid (EAA) receptors in enhanced responses of spinal dorsal horn neurons to mechanical stimuli seen in neuropathic rats.

Neuropathic rats were made by an unilateral ligation of L5-6 spinal nerves. Activity of wide dynamic range (WDR) lumbar dorsal horn neurons evoked by brush and pinch stimulation of the receptive field (RF) was recorded, and effects of iontophoretically applied EAA receptor antagonists on mechanically evoked responses were tested. Comparison was made between neuropathic and normal rats.

Both brush-evoked and pinch-evoked responses of WDR neurons were enhanced in neuropathic rats, as compared to in normal rats. Enhanced brush-evoked response was suppressed by AMPA, non-NMDA antagonist, whereas enhanced pinch-evoked response was suppresed by AP-5, NMDA antagonist. No suppression by either antagonist was observed in normal WDR neurons.

The results implicate that enhanced response to non-noxious mechanical stimulus is mediated by non-NMDA receptor whereas that to noxious one is by NMDA receptor in this rat model of peripheral neuropathy. (Supported by a Grant from KOSEF)

645.4

NMDA RECEPTORS INVOLVEMENT IN MORPHINE BUT NOT OXOTREMORINE-INDUCED ANALGESIA. V. Cestari, C. Rossi-Arnaud*° and F. Pavone^. Università "La Sapienza", Dip. di Genetica e Biologia Molecolare, 00185 Roma, Italy. °Università "La Sapienza", Facoltà di Psicologia, 00185 Roma, Italy, ^Ist. di Psicobiologia e Psicofarmacologia (C.N.R.) - 00198 Roma, Italy.

Recent results show an involvement of the glutamatergic system in modulating pain responses in animals. A number of data show that the activation of the NMDA receptors plays an important role in chronic pain and spinal nociceptive processes.

Two groups of experiments were carried out in order to investigate if NMDA receptors were involved in the well known analgesic response induced by the stimulation of the cholinergic or the opioid systems. In a first group of experiments, CD1 mice were injected with the non-competitive NMDA receptor antagonist MK-801 (0.075, 0.1, 0.125, 0.15 mg/kg, i.p.) and 15 minutes later with vehicle or oxotremorine (0.03 mg/kg, i.p.). After 15, 30 and 60 minutes from the second injection, they were tested in the tail-flick test. The results showed no significative effects on pain sensitivity of mice injected with MK-801. Moreover, MK-801 did not significantly modify the analgesic effect of oxotremorine. On the other hand, the results of a second group of experiments showed that the antinociceptive response induced by the administration of morphine (5, 10 mg/kg , i.p.) was potentiated by MK-801 (0.075, 0.1, 0.125, 0.15 mg/kg, i.p.). The present results support the hypothesis that the glutamatergic system is involved in the antinociceptive effects induced by the stimulation of the opioid system, while they seem to rule out that in our experimental conditions NMDA receptors mediate the analgesic response due to the cholinergic activation.

645.5

NMDA ANTAGONIST, MK-801, BLOCKS THE DEVELOPMENT OF TOLERANCE TO SWIM STRESS-INDUCED ANALGESIA IN MICE. John M. Owen*, Michael W. Haydel and Anthony L. Vaccarino, Department of Psychology, University of New Orleans, LA 70148.

Several lines of evidence indicate a critical role of the *N*-methyl-D-aspartate (NMDA) receptor in the development of tolerance to morphine-induced analgesia. The present study examined the involvement of the NMDA receptor in the development of tolerance to swim stress-induced analgesia (SSIA). To examine the development of tolerance to repeated stress, male Swiss mice were swum individually for 3 minutes daily in water maintained at 32°C for 15 days. A control group was swum on day 1 and day 15 day only. Pain sensitivity was assessed on day 1 and day 15 in the hot-plate test (56°C) prior to (baseline) and after swim stress. The latency to hind-paw flick was measured and a cut-off period of 60 seconds was imposed to avoid tissue damage. To examine the role of the NMDA receptor, mice were injected with either MK-801 (0.075 mg/kg, i.p.) or saline 15 minutes prior to swimming on days 1-14. Control mice also received daily injections of MK-801 or saline. Repeated stress was found to produce tolerance to SSIA. Pretreatment with MK-801 prevented the development of tolerance to SSIA. These results suggest that tolerance to SSIA is mediated in part by the NMDA receptor. This research was supported by a LSU Neuroscience Incentive Grant.

645.6

N-METHYL-D-ASPARTATE AND NEUROKININ RECEPTORS ARE CRITICAL FOR THE INDUCTION OF LONG-TERM POTENTIATION OF C-FIBER-EVOKED FIELD POTENTIALS IN RAT SPINAL DORSAL HORN. J. Sandkühler, X.-G. Liu and M. Zimmermann*. II. Physiologisches Institut, Universität Heidelberg, 69120 Heidelberg, FRG.

Long-term potentiation (LTP) of C-fiber-evoked field potentials in spinal dorsal horn may underly the prolonged central changes of nociception. In this work the roles of N-methyl-D-aspartate (NMDA) and neurokinin (NK1 and NK2) receptors for the induction of LTP were evaluated by superfusion of rat spinal cord with selective agonists and antagonists of NMDA and neurokinin receptors. Superfusion of spinal cord with NMDA receptor antagonist D-(-)4-(3-phosphonopropyl)piperazine-2-carboxylic (D-CPP, 500 nM) for 30 min before and for 30 min after conditioning tetanic nerve stimulation had little effect on the amplitude of C-fiber-evoked potentials but completely blocked LTP in all 5 rats tested. Superfusion of spinal cord with NMDA (1 μM, 10 μM or 50 μM) induced LTP in only 2 out of 8 rats. Superfusion of the spinal cord with the NK1 receptor antagonist RP 67580 at 5 μM inhibited the amplitudes of C-fiber-evoked potentials by 60% but had no effect at 1 μM. NK2 receptor antagonist SR 48968 at 100 nM depressed the amplitudes of C-fiber evoked field potentials by 80% but had no effect at 10 nM. The superfusion of spinal cord with RP 67580 (1 μM) performed for 30 min before and for 30 min after tetanic stimulation blocked the induction of LTP completely in all 5 rats tested. Superfusion with SR 48968 (10 nM) blocked LTP in 5 out of 7 rats tested. The superfusion of spinal cord with substance P at 1 μM, 10 μM and 100 μM never induced LTP in 5 rats tested. In conclusion, the activitation of NMDA or neurokinin receptors is necessary but not sufficient to induce LTP of C-fiber-evoked field potentials in rat spinal dorsal horn. Supported by the Deutsche Forschungsgemeinschaft.

645.7

CONTRIBUTIONS OF NMDA RECEPTORS TO HYPERALGESIA INDUCED BY INJECTION OF MUSTARD OIL INTO THE ANKLE JOINT OF THE SPINALIZED RAT. E. Silva, C.L. Cleland* and G.F. Gebhart. Depts of Pharmacology, and Physiology and Biophysics, Univ. of Iowa, Iowa City, IA 52242 and Dept of Physiology, Universidad de los Andes, Merida, Venezuela.

Hyperalgesia is a pathological state in which noxious stimulation evokes greater amounts of pain. Numerous studies have implicated NMDA receptors *in experimental models of hyperalgesia*. The goal of these experiments was to determine if NMDA receptors preferentially mediate hyperalgesic nociception over acute nociception in a spinal model of hyperalgesia.

Rats were anesthetized with pentobarbital (45 mg/kg). Their spinal cords were transected at T8-T9, an electrode was placed on the sciatic nerve, and either an intrathecal catheter was inserted at T8 and guided to L1-L2 or a catheter was implanted in the jugular vein. The following day, hyperreflexia was induced by injection of 10 μl of mustard oil into the ankle joint, which caused a rapid onset, long lasting facilitation of the flexion withdrawal reflex elicited by C-fiber intensity (10 mA) electrical stimulation of the sciatic nerve and measured by recording the EMG in the hamstring muscles. NMDA antagonists, APV (i.t.) and ketamine (i.v.), were administered 21 minutes following mustard oil injection or, for control experiments, in the absence of mustard oil injection into the ankle.

APV (0.1-100 nmoles) and ketamine (0.05-10 mg/kg) dose-dependently inhibited both the hyperreflexive and the baseline responses. The ED_{50} for APV was approximately 20 nmoles for both hyerreflexia and baseline responses, although the ED_{50} for ketamine was slightly less for hyperreflexive (~2 mg/kg) than for baseline (~5 mg/kg) responses. These results demonstrate that NMDA receptors are involved in both acute and mustard oil-induced hyperreflexive nociception in spinalized rats.

645.8

MODULATION OF SPINAL VISCERAL NOCICEPTIVE TRANSMISSION BY SPINAL NMDA RECEPTOR ACTIVATION IN THE RAT - R. Kolhekar* and G. F. Gebhart, Dept. of Pharmacology, University of Iowa, Iowa City, IA 52242.

Modulation of responses of L_6-S_2 spinal neurons to colorectal distention (CRD; 20-80 mmHg, 30s) was examined following activation of spinal NMDA receptors in pentobarbital-anesthetized rats. Neurons were recorded extracellularly and drugs (0.1-1 pmoles) were administered by pressure ejection from a multibarrel-electrode assembly. Administration of NMDA as well as D-serine, but not saline, in the vicinity of neurons responsive to CRD produced significant increases in the magnitude of neuronal responses to CRD as well as neuronal discharges after the termination of CRD. NMDA also increased the gain of the stimulus intensity-encoding functions of neurons to CRD and lowered thresholds for neuronal responses to CRD. Convergent cutaneous receptive fields of viscerosomatic neurons responsive to CRD also showed expansion after administration of NMDA or D-serine. All of the above facilitatory effects were produced within 30s-4 min of NMDA or D-serine administration and were blocked by coadministration of D-APV or 7-CK, respectively. Repeated high-intensity electrical stimulation (10 mA, 30s, 1 Hz) in convergent cutaneous receptive fields of neurons responsive to CRD produced a facilitation of neuronal responses to subsequent noxious CRD in a D-APV-sensitive manner.

Taken together, these results demonstrate that excitatory amino acids acting at spinal NMDA receptors enhance spinal transmission of visceral nociceptive input. Spinal NMDA receptors are therefore implicated in the generation of hyperexcitability phenomena manifest as visceral hyperalgesia, allodynia and expanded referral of visceral pain.

645.9

2-AMINO-5-PHOSPHONOVALERIC ACID(AP5) BLOCKS COLORECTAL SENSITIZATION BY TURPENTINE. Y.Ide*, Y. Maehara, S. Tsukahara, L. M. Kitahata, and J.G. Collins. Anes. Dept., Yale University School of Medicine, New Haven, CT 06510

The aim of this study was to examine possible involvement of NMDA receptors in the development of the inflammatory-induced sensitization of the colon by turpentine.

Under pentobarbital anesthesia (40 mg/kg IP), Sprague-Dawley rats were prepared with a laminectomy from T12 to L1 for intrathecal administration of AP5. Physiological parameters were maintained within normal limits. Animals were divided into two groups, control group with 50 μl of saline(NS) and AP5 group(AP5) with 10 mM AP5 in 50 μl saline. Visceromotor response threshold to CRD was determined during control study. After the baseline study, AP5 or saline was administered, and 5 min later VMR threshold was determined. Then 1 ml of 25% turpentine was injected rectally. VMR thresholds were determined every 5 min thereafter for the remaining 90 min. ANOVA and t-test were used for statistical analysis. Turpentine treatment in the saline group resulted in a significant decrease in VMR threshold over the 90-min observation period. In contrast, animals pretreated with AP5 showed no such reduction in VMR threshold during the 90-min observation period. These results suggest that inflammatory disorders of the bowel induced by turpentine produce sensitization involving spinal sensory mechanisms mediated NMDA receptors. (Supported by: NIH Grant NS-09871)

645.10

ADRENAL MEDULLARY TRANSPLANTS ATTENUATE SPINAL CORD cGMP PRODUCTION IN RESPONSE TO PERIPHERAL NERVE INJURY. J.B.Siegan*,A.T.Hama, and J.Sagen. Dept of Anatomy and Cell Biology, Univ. of Illinois at Chicago Medical Center, Chicago, IL 60612.

NMDA receptor activation has been shown to result in a Ca^{2+} dependent increase in cGMP through the production of nitric oxide. In the spinal cord, this cascade of events has been implicated in the persistence of pathological pain following injury. Previous studies in our laboratory have demonstrated that the transplantation of adrenal medullary tissue into the spinal cord subarachnoid space can alleviate chronic pain in animal models. The purpose of this study was to determine whether adrenal medullary transplants act via attenuation of cGMP production in spinal nociceptive regions. Neuropathic pain was induced by unilateral constriction of the sciatic nerve. Two weeks following nerve injury, rats were transplanted with either adrenal medullary or control striated muscle tissue in the spinal subarachnoid space. One week after transplantation, dorsal spinal cord segments (L4-L5, the region of sciatic nerve innervation) were dissected and assayed for cGMP using routine radioimmunoassay. Results demonstrated a marked increase in cGMP levels in animals with peripheral nerve ligation, ipsilateral to the chronic constriction injury. The elevated cGMP levels were also found in nerve ligated animals with control transplants. However, chromaffin cell transplants reduced cGMP below basal in nerve injured and intact (non-lesioned) animals. These results demonstrate that cGMP production can be significantly reduced via adrenal medullary transplants, paralleling the transplant-mediated attenuation of chronic pain. In summary, the results suggest that adrenal medullary transplants may intervene in the cascade of events initiated via the activation of NMDA receptors in pathological pain. Supported by NS25054.

645.11

INTERACTION BETWEEN SPINAL NEOSTIGMINE AND CLONIDINE IN SHEEP: ROLE OF NITRIC OXIDE. C TONG*, Z XU, JC EISENACH, The Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1009

Several lines of evidence suggest that α_2-adrenergic agonists produce analgesia after spinal injection in part by causing acetylcholine (ACh) release. As such, spinal injection of the cholinesterase inhibitor, neostigmine, has been shown to enhance spinal clonidine-induced antinociception in animals. Other data suggest the antinociceptive effects of spinally released ACh are mediated in part by nitric oxide (NO). The current study examined the role of NO in the clonidine-neostigmine interaction in sheep. Following approval from the Animal Care and Use Committee, 7 sheep were prepared with chronic lumbar intrathecal (i.t.) catheters. Dose responses to analgesia (foreleg withdrawal to noxious mechanical stimulus) were obtained to i.t. clonidine, i.t. neostigmine, their combination, i.t. n-methyl-l-arginine (NMLA), i.t. NMLA plus clonidine, and i.t. NMLA plus clonidine and neostigmine. Treatments were compared by 2-way ANOVA on the dose responses and by 1-way ANOVA on the calculated ED50 for each treatment, with $P<0.05$ considered significant. I.t. clonidine produced dose-dependent antinociception, whereas i.t. neostigmine and NMLA were without effect. Neostigmine significantly enhanced clonidine antinociception. NMLA did not affect clonidine-induced antinociception, but blocked the interaction between clonidine and neostigmine. These data agree with previous data in sheep that spinally administered α_2-adrenergic agonists cause antinociception, whereas there is no evidence of chronic spinal cholinergic tone, such that spinal neostigmine alone causes antinociception. Similarly, lack of effect of NMLA alone on antinociception suggests no chronic release of NO in the unstimulated state.

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645.12

EFFECTS OF NITRIC OXIDE SYNTHASE INHIBITORS ON THE OXOTREMORINE-INDUCED ANALGESIA. F.Pavone*, H.Machelska° and B.Przewlocka° Inst. Psychobiology and Psychopharmacology, CNR, Roma, Italy. ° Inst. Pharmacology, PAN, Krakow, Poland.

Recently it has been suggested that nitric oxide could play an important role in peripheral and central cholinergic analgesia. The present research was designed to find out whether the oxotremorine-induced antinociception was mediated by nitric oxide. In a first set of experiments it has been observed that the intraperitoneal injection of the inhibitors of nitric oxide synthase, N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine (N-ARG), in doses of 10 and 20 mg/kg, had no effect on the thermal nociceptive threshold of male CDI mice. When administered before oxotremorine (0.005, 0.01, 0.02, 0.03mg/kg), L-NAME and N-ARG significantly potentiated the dose-dependent analgesic effects of the muscarinic agonist. In a second set of experiments intrathecal administration in rats of Wistar strain of L-NAME (50, 400 µg/5µl) did not change the nociceptive threshold, but dose-dependently potentiated the effects of oxotremorine injected i.p.. in a dose 0.02 µg/kg, both in paw pressure and in tail flick tests. The NO donor SIN-1 (10,100µg/5µl), without effects when given alone, antagonized oxotremorine-induced analgesia in both tests used. The obtained data indicate that nitric oxide exerts an inhibitory action on cholinergic-induced analgesia and evidenced the pronociceptive effects of NO in the spinal cord.

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645.13

THE CONTRIBUTION OF NITRIC OXIDE AND SUBSTANCE P RECEPTOR ACTIVATION TO FORMALIN EVOKED SPINAL C-FOS EXPRESSION V. Chapman, J. Buritova, P. Honoré & J.M. Besson*, INSERM U.161, 2 rue d'Alésia, 75014 Paris.

We have investigated the contribution of substance P and nitric oxide (NO) to formalin evoked spinal expression of c-Fos. Both of these receptor systems are strongly implicated in the spinal processing of prolonged, but less so acute, nociception.

Three hours after intraplantar formalin Fos-immunoreactive (Fos-LI) neurons (total expression 175±13 neurons per section) were essentially located both in the superficial (94±6 Fos-LI neurons) and deep (57±6 Fos-LI neurons) laminae of the dorsal horn of L4-L5 segments of the spinal cord. Systemic administration of 7-Nitro indazole (7NI), a selective inhibitor of neuronal nitric oxide synthase, 30 minutes prior to intraplantar formalin, dose relatedly reduced the deep laminae expression of c-Fos. The highest concentration of 7NI (15mg/kg) significantly inhibited the number of Fos-LI neurons in the deep laminae of the dorsal horn (42±8% of control expression, $p<0.05$), in addition there was a tendency towards a reduction of superficial c-Fos expression.

In the second part of this study the effect of systemic administration of the selective neurokinin 1 receptor antagonist, RP67580 on formalin evoked c-Fos expression was studied. RP67580 dose relatedly reduced the number of formalin evoked Fos-LI neurons in both the superficial and deep laminae of the dorsal horn of the spinal cord, with 1500µg/kg of RP67580 significantly reducing both the superficial and deep laminae expression of c-Fos (24±4% and 35±7% reduction of control expression respectively, $p<0.05$ for both). In contrast 1500µg/kg of RP68651, the inactive isomer of RP67580, did not significantly influence formalin evoked c-Fos expression. Neither 7NI or RP67580 influenced the peripheral formalin evoked inflammation.

These results clearly demonstrate the contribution of both substance P and NO to inflammatory evoked c-Fos expression at the spinal level, however they also illustrate that substance P influences noxious events both in the superficial and deep laminae of the dorsal horn, whereas NO appears to preferentially influence deep laminae neurons. Since the peripheral inflammatory process was not influenced these effects are probably due to a central site of action.

645.14

INVOLVEMENT OF NITRIC OXIDE IN PROSTAGLANDIN $F_{2\alpha}$-INDUCED LONG LASTING ALLODYNIA . Y.Saito*, Y.Kirihara, K.Hara, M.Kaneko, Y.Yamamori and Y.Kosaka, Dept. of Anesthesiol., Shimane Med.Univ., Izumo, Shimane 693 Japan

We have reported that intrathecal (i.t.) prostaglandins (PGs) induce long lasting allodynia (Neurosci. Abst., 1993; Pain, in press). The present study examined the effect of nitric oxide synthase inhibitor (NOSI) on the $PGF_{2\alpha}$-induced long lasting allodynia.

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

Posttreatment with L-NMMA relieved the increase of ASs induced by $PGF_{2\alpha}$ in a dose dependent fashion for 60 min and then ASs returned to the baseline level while saline had no effect on the increase of ASs. Pretreatment with less than 300 µg of L-NMMA temporarily attenuated the increase of ASs induced by $PGF_{2\alpha}$. In most animals, i.t. administration of $PGF_{2\alpha}$ following pretreatment with 1000 µg of L-NMMA did not produce any increases of ASs for 7 days. These results suggest that NO may participate in the initiation of $PGF_{2\alpha}$-induced long lasting allodynia in the spinal cord.

645.15

ANTINOCICEPTION AFTER AN INTRATHECAL INJECTION OF *W*-NITRO-L-ARGININE (L-NAME) IN MICE. A.A. Larson* and K. F. Kitto. Department of Veterinary Pathobiology, University of Minnesota, St. Paul, MN 55455, U.S.A.

The present study was designed to determine the role of nitric oxide (NO) in nociception by assessing the effect of L-NAME, a NO synthase inhibitor, on nociception. Although L-NAME has been found to inhibit hyperalgesia, antinociception following 5 nmoles of L-NAME was not observed at 1 or 6 hours after its injection when tested using the hot plate, tail flick or writhing assays. However, 16 and 24 hr after its injection intrathecally, L-NAME (10 to 100 nmoles) produced a dose-related inhibition of acetic acid-induced writhing behaviors without affecting the latency of response to the thermal stimuli of the hot plate or tail flick assays. Full recovery was observed at 48 hr. L-arginine alone did not alter nociception, however, pretreatment with 120 nmoles of L-arginine completely prevented the antinociceptive effect of 30 nmoles of L-NAME, suggesting that the effect of L-NAME was due to its ability to inhibit NO synthesis. It does not appear that inhibition of NO synthesis persists for 24 hr as L-arginine, injected 30 min prior to nociceptive testing, failed to reverse the antinociceptive effect of L-NAME. The inhibition of writhing by L-NAME was not mimicked by pretreatment intrathecally with either 10 nmoles of methylene blue, an inhibitor of guanylate cyclase, or a dose as high as 300 nmoles of D-NAME, the inactive isomer of L-NAME. Together these data suggest that antinociception observed 24 hr after injection of L-NAME is brought about by a transient inhibition of NO synthesis. These data suggest that NO does not mediate but sustains a normal nociceptive tone along specific pain pathways. *(Supported by NIDA 04090)*

645.16

SEROTONERGIC CELLS, NITRIC OXIDE NEURONS, AND SITES OF MUSCARINIC ANALGESIA COINCIDE IN ROSTRAL VENTRAL MEDULLA OF RATS. Edgar Iwamoto*. Dept. of Pharmacology, Univ. of Kentucky College of Med., Lexington, KY 40536

Muscarinic agonists microinjected into the rostral ventral medulla (RVM) of rats produce antinociception in the hot-plate and tail-flick tests which may be mediated by nitric oxide and cGMP (Iwamoto and Marion, JPET 271:601, 1994). Located at the juncture and vicinity of the gigantocellular reticular nucleus pars *alpha* (GiA) and the lateral paragigantocellular nucleus (LPGi) are neurons containing mRNAs coding for soluble guanylyl cyclase and for neuronal nitric oxide synthase (Iwamoto *et al.* Soc. Neurosci. 19:968, 1993). The present study was designed to test the hypothesis that the muscarinic analgesia mediated by nitric oxide in the RVM is transduced by serotonergic neurons. Complementary oligos to neuronal nitric oxide synthase or serotonin transporter mRNAs were 3'-end labeled using [α-^{33}P]dATP, hybridized to 20 μ sections of rat RVM, and localized by film and emulsion autoradiography. The *in situ* hybridization pattern of cells in RVM containing the serotonin transporter reflected the known locations of B2 and B3 cells, and a portion of labeled B3 neurons coincided with GiA and LPGi neurons labeled by the nitric oxide synthase oligo. These results are consistent with the hypothesis that muscarinic analgesia may be mediated by a m1/nitric oxide/cyclic GMP transducer apposed to descending serotonergic pathways originating from the rat RVM. This could explain why cholinergic analgesia is blocked by intrathecal administration of serotonin receptor blockers (Iwamoto and Marion, JPET 265:777, 1993). Supported by NIH NS 28847

645.17

Capsaicin Evokes the Release of i-CGRP from Dorsal Horn Slices in a Nitric Oxide Dependent Manner. Mary G. Garry,* Ph.D. Department of Anesthesiology, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235

Sodium nitroprusside, a nitric oxide (NO) donor, evokes the release of neuropeptides from primary afferent neurons in dorsal horn spinal cord slices in an NO-dependent and NO-independent manner (*Garry et al., J. Neurosci. 14:4329*). To further elucidate whether NO is involved in peptide release from nociceptive neurons, we examined whether the effects of capsaicin, [CAP; a neurotoxin which selectively stimulates nociceptive primary afferent neurons and evokes the release of peptides such as calcitonin gene-related peptide (CGRP)] are dependent upon an NO mediated mechanism. Rat spinal cords were ejected by hydraulic extrusion. The dorsal horn of the lumbar enlargement was isolated and placed into chambers which were superfused with oxygenated Kreb's buffer (pH 7.4) for a 60 min equilibration period. Following the equilibration period, tissues were superfused with Kreb's buffer (veh; n=5) or CAP (1μM; n=5) to evaluate the effect of CAP on CGRP release. To determine whether CAP evokes CGRP release via an NO dependent mechanism, tissues were superfused in the presence of CAP alone (1μM, n=7) or L-NAME (200 nM; a nitric oxide synthase inhibitor, n=4) prior to and during stimulation with CAP. The amount of immunoreactive CGRP (iCGRP) in the superfusates was assessed by RIA. Raw data were analyzed using ANOVA. Basal levels of i-CGRP release were 1,462.2±170 fmol/g/6min whereas 1μM CAP significantly increased i-CGRP release to 13,473.2± 970.8 fmol/g/6min ($F_{1,9}$=135.49; p<0.0001). In the presence of L-NAME, CAP-evoked i-CGRP release was significantly reduced to 4,793.7±687.2 ($F_{1,9}$=38.14; p<0.001). In support of these data, we also observed that CAP evokes a dose-dependent (1-30μM) increase in citrulline production in dorsal horn slices. Collectively, these results indicate that CAP evokes the release of i-CGRP from dorsal horn slices via an NO dependent mechanism. These data suggest that the NO dependent hyperalgesia which has been observed at the level of the spinal cord, maybe due to the effect of NO on the release of certain neuropeptides. These studies are supported by the Sid W. Richardson Foundation.

645.18

NEUROPATHIC CHANGES IN MICE AFTER INTRATHECAL APPLICATION OF L-NAME AND/OR STRYCHNINE. T. M. Laughlin*[1], K. F. Kitto[1], R. P. Yezierski[2], G. L. Wilcox[1,3]. [1]Dept of Pharmacology and [3]Graduate Program in Neuroscience, Univ. of Minnesota, Minneapolis, MN 55455. [2]Dept. of Neurological Surgery and The Miami Project, Univ. of Miami, Miami, FL 33136.

N^{ω}-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor, has been used to study the involvement of nitric oxide in acute and chronic nociception of rodents. Injections of L-NAME in rat dorsal horn produce delayed spinal cord lesions associated with hyperalgesia and allodynia. The involvement of neuronal nitric oxide in activity-dependent cerebrovascular control suggests that these lesions result from ischemia and excitotoxicity. We tested whether intrathecal (i.t.) L-NAME and/or strychnine, a glycine receptor antagonist that produces acute allodynia, would synergize in this lesioning process.

750 mM L-NAME, 325 mM L-NAME + 54 nmol strychnine, 750 mM D-NAME, 54 nmol strychnine, or saline were injected i.t. in male ICR mice. L-NAME was given as a pretreatment one hour before saline or strychnine. The second injection was followed 10 min later by an intraplantar injection of formalin (4%) to drive the dorsal horn. Hyperalgesia was tested by the hot plate (52.5°C) and hot water tail flick (53°C) tests. Cold allodynia was tested by multiple applications of 10 µl acetone to each hind paw. Behavioral tests were administered prior to treatment and repeated on days 4, 7, 14, and 15. Fifteen days after treatment, mice were challenged with i.t. morphine (0.15, 1.5, 15 nmol) to determine opioid sensitivity in the hyperalgesic state. Finally, mice were perfused transcardially with 10% formalin, the spinal cords removed by laminectomy, sectioned at 50 µm and stained with cresyl violet.

Two weeks after i.t. L-NAME, mice showed hyperalgesia in the tail flick test and allodynia in the acetone test. Coadministered strychnine enhanced responses only in the acetone test. L-NAME-treated mice were resistant to i.t. morphine in the tail flick test, whereas mice given L-NAME plus strychnine showed dose-related morphine antinociception. Cold allodynia was inhibited by morphine, although not in a dose-related manner. These behavioral indicators of enhanced nociception were not accompanied by pronounced lesions like those seen in rats, suggesting a strong species dependence. The behavioral findings suggest that similar plastic changes take place in mice and rats after high doses of NOS inhibitors and neuronal activators. (Supported by KO2-DA-00145 & DA-04274 to GLW, and NS28059 to RPY)

RETINAL FUNCTION

646.1

DEVELOPMENT OF COUPLING BETWEEN DIRECTION-SELECTIVE RETINAL GANGLION CELLS. D. I. DeBoer, W. H. Baldridge* and D. I. Vaney. Vision, Touch & Hearing Research Centre, The University of Queensland, Australia.

The On-Off direction-selective (DS) ganglion cells in rabbit retina comprise four distinct subtypes, which orthogonally code the direction of image motion. Each subtype provides complete coverage of the retina, with little overlap between neighbouring cells of the same subtype. This study investigated the postnatal development of the territorial organisation and the coupling patterns of the On-Off DS cells. Following pentobarbitone overdose, the eyes were enucleated, the mounted retinae superfused with Ames medium, and DAPI-labelled ganglion cells were injected with Neurobiotin. By postnatal-day 10, the presumptive DS cells had an adult-like organisation, with only about 40% of injected cells showing strong homotypic tracer coupling to a territorial array of DS cells. At postnatal-day 5 and earlier, however, most injected cells appeared to be coupled not only to a regular array of neighbouring DS cells, but also to one or more arrays of overlying DS cells. Consequently, coupled somata were located inside the dendritic field of the injected cell, unlike the adult pattern, producing multiple dendritic-field overlap between coupled cells. Thus, there appears to be both 'homo-subtypic' and 'heterosubtypic' coupling between the developing On-Off DS cells, with subsequent uncoupling to produce the adult pattern. The immature coupling pattern appears to be more complex than would be required to simply shape the territorial organisation of the DS ganglion cells; the heterosubtypic coupling may thus serve additional processes requiring coordinated communication between the different subtypes of DS cells. Moreover, our findings indicate that the subtypes of On-Off DS cells are morphologically established before ganglion cells are visually responsive, with important implications for how direction selectivity develops.

646.2

DIRECTIONAL SELECTIVITY IN RETINAL GANGLION CELLS IS ABOLISHED BY NEOMYCIN. R. J. Jensen*. Southern College of Optometry, Memphis, TN 38104.

Findings from several studies suggest that the antibiotic neomycin may block voltage-activated P- or Q-type Ca^{2+} channels. In a recent study (Jensen, *J. Neurophysiol.*, In press) it was found that the Q-type Ca^{2+} channel blocker ω-conotoxin MVIIC abolishes directional selectivity in ON/OFF directionally selective ganglion cells in the rabbit retina. This effect was not observed with either T-, L-, N-, or P-type Ca^{2+} channel blockers. The present study was undertaken to see if neomycin could abolish directional selectivity in these ganglion cells.

Extracellular recordings were made from ON/OFF directionally selective ganglion cells in superfused rabbit retinal strips. At 480-800 µM, neomycin abolished directional selectivity in these cells (n = 7) by bringing out a response to movement in the null direction that was similar in magnitude to the response to movement in the preferred direction. The effect of neomycin was rapidly reversible upon washout. Similar findings were obtained with gentamicin, streptomycin, and tobramycin but only at concentrations greater than 1000 µM. In conclusion, neomycin and other aminoglycoside antibiotics appear to block ω-conotoxin MVIIC-sensitive Ca^{2+} channels in the retina. (Supported by NIH EY07318)

646.3

Linear contribution to directional selectivity in cat retinal ganglion cells. M.H. Rowe*, Neurobiology Program, Ohio University, Athens, OH 45701.

Directionally selective simple cells in cat visual cortex often have receptive field profiles that are oriented in the space-time plane, and this form of spatio-temporal coupling provides the basis for their directional selectivity. Recent examination of phasic W-cells in the cat retina (Rowe and Palmer, 1995) revealed the presence within many of their receptive fields of multiple subregions that can be separated in either space or time. Some of these cells are directionally selective and it is possible that these cells could show the same form of spatio-temporal coupling that has been described in cortical simple cells.

Linear spatio-temporal response profiles were generated for a number of directionally selective W-cells using a reverse correlation procedure. The spatial axis of the profile was either aligned with the axis of directional preference or orthogonal to it. In most cases, these response profiles were spatio-temporally separable, indicating that linear mechanisms did not contribute to the cell's directional selectivity. In a few cases, weak spatio-temporal coupling was observed when the spatial axis was aligned with the axis of directional preference, i.e., the response profiles showed a space-time orientation that predicted the cell's directional preference. However, these contributions were generally small. The sample of directionally selective cells also included one suppressed-by-contrast cell whose spatio-temporal response showed a clear orientation consistent with its directional preference. These data suggest that linear spatio-temporal summation does not contribute significantly to directional selectivity in cat retinal ganglion cells.

Supported by EY10677 and by funds from OUCOM.

646.4

ANALYSIS OF DECOUPLING EFFECT OF CYCLIC GMP ON GAP JUNCTIONS BETWEEN RETINAL NEURONS, USING PROTEIN KINASE INHIBITORS. E.-I. Miyachi* and C. Nishikawa. Dept. of Physiol., Fujita Health Univ. Sch. of Med., Toyoake, Aichi 470-11, JAPAN

Intracellular injection of cyclic GMP or soluble guanylate cyclase activators, such as nitric oxide (NO) and arachidonic acid, closed gap junctions between horizontal cells in carp and turtle retinas (Miyachi et al., NeuroReport 1: 107-110, 1990; Miyachi et al., NeuroReport 5: 485-488, 1994). To determine whether the blocking effect of cyclic GMP on the gap junctions was through the action of cyclic GMP-dependent protein kinase, we examined the status of dye-coupling with Lucifer Yellow CH among luminosity-type horizontal (H1) cells in the carp retina, by injection of protein kinase inhibitors into the cells. Dye-coupling among horizontal cells was observed after the intracellular injection of the mixture of cyclic GMP and a cyclic GMP- and cyclic AMP-dependent protein kinase, A-3 (N-(2-aminoethyl)-5-chloronaphthalene-1-sulfonamide hydrochloride). Dye-coupling was observed also after the injection of the mixture of cyclic GMP and cyclic GMP-dependent protein kinase inhibitor peptide (Arg-Lys-Arg-Ala-Arg-Lys-Glu) into the horizontal cell, indicating that the inhibitor suppressed the decoupling effect of cyclic GMP. The same kinase inhibitor did not suppress the decoupling effect of cyclic AMP on the gap junctions. On the other hand, a single cell was seen fluorescing after the injection of the mixture of cyclic GMP and cyclic AMP-dependent protein kinase inhibitor peptide (Arg-Gly-Try-Ala-Leu-Gly). These findings suggest that the blocking effect of cyclic GMP on gap-junctional intercellular communication among horizontal cells is through the action of cyclic GMP-dependent protein kinase, and that cyclic GMP modulates the gap junctions in a manner independent of the well-known dopamine/cyclic AMP pathway in the horizontal cell.

646.5

PHYSIOLOGICAL PROPERTIES OF AXON BEARING WIDE FIELD AMACRINE CELLS IN THE RABBIT RETINA. W. R. Taylor and H. Wässle*, Max-Planck-Institut für Hirnforschung, D-60528 Frankfurt, Germany.

Axon bearing wide field amacrine cells in the rabbit retina have a central sparse dendritic arbor, from which several axon-like processes emerge. The axons are generally straight, randomly oriented and can be several millimeters in length (Vaney et al., 1988[1]). We address two questions important for the physiology of these cells. 1) Do the cells generate action potentials? 2) How large is the receptive field?

Patch-clamp recordings were made from cells in the ganglion cell layer of an isolated, dark adapted, flat-mounted rabbit retina preparation. Light stimuli were generated on a computer monitor that was imaged onto the photoreceptor layer through the microscope condenser. Cell morphology was revealed by neurobiotin staining

Action potentials were elicited during depolarization. The receptive field centre had a diameter similar to that of the dendritic arbor. There appeared to be an excitatory central region and a larger inhibitory surround. The cells generated transient excitatory currents in response to a light step. The excitatory current decayed with a time constant of about 50ms.

These cells receive input across a relatively small dendritic arbor and distribute their output via action potentials across a much wider axon terminal system. The mosaic of these cells could provide a rapidly responding, global measure of the luminance across the retina.

1. Vaney, D.I., Peichl, L. and Boycott, B.B. *Proc. R. Soc. Lond. B* **235**:203.

646.6

CONTRIBUTION OF THE BETA GANGLION CELL SURROUND TO INFORMATION PROCESSING. Y.Tsukamoto[1]*, R.G. Smith[2] and P. Sterling[2] Hyogo Col. Med., Hyogo, 663 Japan[1] Dept. Neurosci., U. Penn, Phila.PA. 19104[2]

Spatial correlation in a natural scene is exponential. Therefore, cone signals contributing to a receptive field center are strongly correlated and those contributing to the surround are weakly correlated. Summing the center signals improves the signal/noise ratio (S/N) compared that of a single cone (Tsukamoto et al, '90), but the effect of subtracting the surround signals is unknown. We compared the S/N improvement (I) for the center only and for center-surround. The computation incorporated known cone and beta ganglion cell densities and receptive field parameters, plus scenes of different spatial frequency composition (characterized by the space constant, λ) When high frequencies are abundant (λ<0.1mm), S/NI is slightly greater for the center-surround than for the center only and correlations between neighboring cells are slightly enhanced. When low frequencies are abundant (λ>0.1mm), S/NI for center-surround is less than for the center only and correlations between neighboring cells are reduced. The effects of the surround on S/NI are nearly constant with eccentricity, due to the relative expansion of the center. S/NI is always considerably higher for peripheral than for central cells. The overall design improves encoding by central beta cells of high spatial frequencies (preserved by smooth pursuit eye movements) and encoding by peripheral beta cells of lower spatial frequencies (arising from blur due to smooth pursuit).

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646.7

CONTRAST GAIN CONTROL IN THE LOWER VERTEBRATES RETINAS. J.-L. Wang and K.-I. Naka*. Dept. of Ophthalmol. and Dept. Physiol. & Biophysics., NYU Med. Ctr., New York, NY 10016.

Control of contrast sensitivity was studied in the retinas of the channel catfish and that of the kissing gourami. The former preparation is dominantly monochromatic and the latter is bichromatic. Recordings were made from horizontal, amacrine, and ganglion cells and results were analyzed by Wiener analysis, in which the kernels represent the contrast (incremental) sensitivity. Modulation responses from horizontal cells are linear, in that the waveform and amplitude of first-order kernels are independent of the depth of modulation. In the N (sustained) amacrine and ganglion cells, contrast sensitivity was low for a large modulation input and was high for a small modulation input. In most of the cells, the control of contrast gain did not affect the dynamics of the response since the waveform of the first-order kernels remained unchanged when the contrast sensitivity increased more than 5-fold. An analysis of a cascade of the Wiener type shows that the control of contrast sensitivity in the proximal retinal cells can be explained by assuming the presence of a (static) saturation nonlinearity. Such a nonlinearity must exist somewhere between the horizontal cells and the amacrine cells. The functional implications are as follows: 1) neurons exhibit greater sensitivity to input of lower contrast, 2) saturation of a neuronal response can be prevented because of the lower sensitivity in the case of an input with large contrast, and 3) over a large range of modulation depths, the amplitude of the response remains approximately constant.

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646.8

CONTRAST FLICKER ERG RESPONSES TO CONE-ISOLATING STIMULI. David H. Brainard, Jack B. Calderone, and Gerald H. Jacobs*. Department of Psychology and Neuroscience Research Institute, UC Santa Barbara, 93106

The potentials measured by the flicker ERG originate at multiple sites in the retina. To exploit the ERG to understand the flow of information through the retina, it is necessary to develop techniques that allow one to distinguish activity at individual sites or in particular pathways. To this end, we have recorded flicker ERG responses to stimuli whose spectral properties isolate individual classes of cone photoreceptors. Stimuli were presented on a computer-controlled color monitor. The color of the stimulus field was modulated temporally around a fixed mean level of illumination. Control software allowed us to specify independently the modulation contrast seen by human long, middle, and short wavelength sensitive cones. ERG responses were recorded both to cone isolating stimuli and to stimuli that modulated multiple cone classes in various combinations. We began by measuring the contrast response function for isochromatic flicker. As others have found, the shape of this function depends heavily on temporal frequency. At 18.75 Hz, we recorded reliable responses to cone-isolating flicker for all three classes of cone. At low contrasts, the contrast response functions for all three cone classes are linear. We used a flicker-photometric paradigm to assess how signals from the three cone classes are combined. In this technique, the responses to various cone-isolating stimuli and combinations of cone-isolating stimuli are balanced against the responses generated by an interleaved reference stimulus. Two subjects gave differing results: for one subject the rule of combination was close to linear, in a second subject we observed strong spectral opponency between the long and middle wavelength sensitive cones.

646.9

DYNAMICS OF ADAPTATION TO CHANGING SPATIAL STRUCTURE IN THE TIGER SALAMANDER RETINA. S.M. Smirnakis*[,1], M.J. Berry[2], D.K. Warland[2], W. Bialek[3], and M. Meister[2]. [1]Physics Dept and Medical School, Harvard University; [2]MCB Dept, Harvard University; [3]NEC Research Institue, Princeton, NJ.

The neural code employed by the retina is known to adapt dynamically to changes in the statistics of the visual scene, such as the mean intensity and temporal contrast. Here we focus on how the retina adapts to changes in the spatial correlation length of the image. Isolated salamander retinae were stimulated with flickering checkerboard patterns and ganglion cell spike trains were recorded extracellularly with a multi-electrode array. The intensity of each checker was chosen randomly every 30 ms from a Gaussian distribution of fixed mean (photopic range) and root-mean-square contrast (24% of the mean). Every 100 s, the size of the checkers was changed, ranging from ~1/4 of a ganglion cell receptive field center to the full field. We observed adaptation to these transitions in both the ganglion cell firing rate and the reverse correlation to the stimulus.

The steady-state firing rates of ganglion cells depended on checker size; larger receptive field centers correlated with maximal responses for larger checkers. After a transition in checker size, the firing rate approached steady-state exponentially. When alternating between full field and 100 µm checker stimulation, the rate of 25 out of 45 ganglion cells decreased with adaptation for both transitions; the decline ranged from 5% to 100% of the final value, with time constants clustered around 10 s. This suggests that there are at least two independent sites of adaptation in the retina. Because the stimuli all had the same local statistics, it is unlikely that either site is in the photoreceptors. Furthermore, changes in spatial correlation length also altered the temporal aspects of the neural code: reverse correlation showed that the response latency of both the center and the surround decreased for larger checkers.

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646.10

NEURAL CODING IN THE *LIMULUS* VISUAL SYSTEM

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The *Limulus* eye samples its visual world with a relatively small number of retinal receptors and optic nerve fibers (~1000). High environmental noise further limits the information capacity of the optic nerve, and yet male *Limulus* can detect females both day and night. Is the eye tuned to detect mates? We address this question by measuring the spatiotemporal transfer function (STTF) of the eye, defining the spectral properties of the environment, and developing a computational model of the eye to explore the information content of the optic nerve.

We measured STTFs of single optic nerve fibers from submerged animals using an sinewave generator-recorder system (VENUS). We also recorded optic nerve responses from behaving animals in their natural environment. We then computed "neural images" for both sinusoidal patterns and natural underwater scenes recorded with a camera mounted on the behaving animal By analyzing the "neural images" and conducting parametric studies of the model eye, we studied the eye's tuning properties and its ability to cope with environmental noise.

We found that computed "neural images" accurately represent recorded optic nerve activities and thus validate the model. We also found that the structural and physiological properties of the eye are better tuned to the spatiotemporal features of a female than to those of environmental noise.

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646.11

ANATOMICAL IDENTIFICATION OF SHORT WAVELENGTH SENSITIVE (S) CONE INPUT TO H1 AND H2 HORIZONTAL CELLS IN MACAQUE MONKEY RETINA. A.K. Goodchild, T.L. Chan and U. Grünert*. Department of Physiology F13, The University of Sydney, N.S.W. 2006, Australia.

In lower vertebrates horizontal cells have selective connections to cones of different spectral types. In primates, the presence of such selectivity remains controversial. In order to address this question anatomically, we studied the connections of H1 and H2 horizontal cells with putative S cones in macaque monkey retina. Horizontal cells were labelled in two ways: 1) An *in vitro* wholemount preparation was used to inject horizontal cells intracellularly with Neurobiotin. 2) In paraformaldehyde fixed retinas horizontal cells were labelled with DiI. The fluorescent DiI label was photoconverted to a permanent reaction product (Sandell and Masland, *J. Histochem. Cytochem., 35:555, 1988*). The retinas were then processed with antibodies against human S cone pigment (JH455, kindly provided by Dr. J. Nathans). The following results were obtained. Labelled S cones made up less than 10% of the cone population; they formed a regular array similar to that described previously (DeMonasterio et al., *Science 213:1278, 1981*). The entire cone including its pedicle was labelled, enabling us to investigate the contacts of S cone pedicles with labelled horizontal cells. Most H1 horizontal cells (34 of 41 cells) did not contact S cones. Labelled dendrites were seen passing over S cone pedicles but not contacting them. In those cases where H1 cells contacted S cone pedicles (7 of 41 cells) comparatively few (always less than 3) dendritic terminal knobs made these contacts. H2 cells (n=4) contacted all S cone pedicles within their dendritic field. Dendrites of H2 cells tended to aggregate at the S cone pedicle. Contact was made by numerous dendritic terminal knobs, whereas contacts with unlabelled pedicles were only sparse. Our anatomical findings suggest that H1 cells receive little or no input from S cones, whereas H2 cells receive input from all cone types. Our results are in good agreement with the recent physiological findings of Dacey and Lee (*ARVO Abstr., IOVS 36:10, 1995*).

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646.12

RECOVERY PHASE OF THE MURINE RETINAL ROD PHOTORESPONSE DETERMINED FROM THE ERG BY A TWO-FLASH PROTOCOL. A.L.Lyubarsky* and E.N.Pugh, Jr. Department of Psychology and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104

The a-wave of the murine ERG reflects the activation phase of the rod photocurrent response, whereas the photoresponse recovery phase is obscured by other components of the ERG. We recorded corneal ERGs of CBA/CAJ mice, dark adapted for 2 hr and anesthetized with a ketamine-xylazine-urethane mixture. Whole-retina stimulation was provided by pairs of light flashes. The amplitude of the a-wave response to the second, saturating flash was used as a measure of the degree of rod recovery from the first. The entire recovery time course from flashes producing 300 - 20,000 photoisomerizations/rod (Rh*/rod) was reconstructed by varying the intervals between flashes. The reconstructed responses were similar to those previously recorded from the photoreceptor layer of isolated rat retina[1]. Over the intensity range 300-5000 Rh*/rod recovery half-times increased linearly with ln(Rh*/rod), with an apparent time constant of 0.3 s.

[1]Penn & Hagins, Biophys. J., 12, 1073-1094.

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646.13

THE LOW-FREQUENCY VOLTAGE NOISE IN THE ROD NETWORK OF THE DARK-ADAPTED FROG. K. Djupsund*[1,2] and T. Hariyama[3]
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Previous studies [e.g. Baylor et al., J Physiol 309, 591-621] have shown that isomerization events in isolated rods occur in the low-frequency spectral range. To see how these results correspond to the noise output in the intact rod network we studied the low-frequency voltage noise in dark-adapted frog (Rana ridibunda) rods and estimated the equivalent isomerization rates.

The study was carried out by intracellular recordings in completely dark-adapted (>15h) eyecups. In addition we verified the pigment composition with HPLC. The IC recordings consisted of flash responses and 200 s. noise periods in darkness and under a background of 1 isomerization per rod*s (=1 Rh*/s), in 10 cases in two temperatures. The spectral analysis was done with 1024 point FFT/Hanning windowing.

The visual chromophore was purely A_1. The amount of equivalent isomerizations in the voltage variance under 1Hz in darkness equalled 0.17±0.10 Rh*/s (n=17) at 10°C and had a Q_{10} of 3.3 (ΔT=4°C, n=10). Our two estimation methods also indicated a significant (36%-54%) non-isomerization induced component in the noise variance, reducing the rates and Q_{10} of the isomerization-induced noise to 0.12±0.07 or 0.069±0.035 Rh*/s and 1.8 (one method only), respectively.

These results indicate that the dark noise levels in the rod network are higher than in isolated rods, but the temperature characteristics are relatively similar. The differences might be explained by e.g. additional processes in the network or a covariance between the noise components.

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646.14

DUPLICITY WITHIN THE ROD MECHANISM OF RAT. F. Naarendorp* Psychol. Dept., Northeastern University, Boston, MA 02115.

Flicker studies on human revealed two separate rod pathways: a slow and a fast pathway (Conner, *JPhysiol, 332, '82*). Rod flicker threshold vs illuminance functions showed a double branch and flicker frequencies of 14-15 Hz appeared invisible over a limited range of retinal illuminance. In this range, a slow and a fast rod signal are thought to interfere destructively with each other and cancel flicker visibility (Stockman et al, *JOSA, 8, '91*). We examined whether a similar duality of temporal function occured in the rod system of rat. Electroretinographic (ERG) responses to square-wave flicker or to sinusoidally modulated light were recorded at the cornea of anesthetized, male, albino rats. Results were as follows: (a) in a plot of CFF vs luminance, CFF increased to about 15 Hz when the luminance reached -2.3 log cd/m^2, then decreased to 11 Hz over the range of -2.0 to -0.8 log cd/m^2. Above -0.5 log cd/m^2 rod CFF rose again reaching 19 Hz at 1 log cd/m^2. (b) flicker amplitude vs luminance curves showed that, up to 0.5 log cd/m^2, response amplitudes to 3-5 Hz flicker increased monotonically. Amplitudes to 7-15 hz flicker first increased then began to decrease at -2.0 log cd/m^2. Responses to 13-15 Hz flicker disappeared completely at -1.5 log cd/m^2 but reappeared at -1.0 log cd/m^2 when amplitudes for frequencies > 7 Hz began to grow again. Above this intensity response amplitudes to 17 and 19 hz flicker were measurable. The phase of the response to 13 Hz flicker at -2.0 log cd/m^2 was shifted by 180° relative to that recorded at -0.5 log cd/m^2. Our data suggest that there is a duality of temporal function within the rod system of rat and it strongly resembles that of human.

646.15

PHASIC AND TONIC RESPONSES IN THE RETINA OF THE PIGEON: DISTRIBUTION AND DIFFERENTIAL CHROMATIC PROPERTIES. J. Mpodozis*, R. Panteon and J-C. Letelier. Departamento de Biología, Facultad de Ciencias, Universidad de Chile. Casilla 653, Santiago, Chile.

The response characteristics of the pigeon's retinal ganglion cells were examined by direct intraretinal extracellular recordings. According to their temporal dynamics, two types of responses, tonic and phasic, were found. Phasic cells are mostly ON/OFF movement sensitive units, while tonic cells show predominantly exclusive ON or exclusive OFF responses, and lack movement sensitivity. Furthermore, phasic and tonic units have different chromatic properties. Phasic cells show very wide action spectra, and 50% of them show clear responses to the movement of a pure chromatic edge in the long-short and long-middle spectral range. On the other hand, tonic cells have narrow action spectra, and 30% of them show well defined inhibitory color opponent surrounds. We also studied the spatial distribution in the visual field of these responses. Phasic responses can be found in the whole retina, but they are more abundant and densely packed in the lateral retina around the lateral fovea. Tonic responses are almost exclusively present in the dorso-temporal retina. Accordingly, we think that these two kinds of ganglion cell responses contribute to generate the differential perceptual properties that the frontal and lateral gaze of birds have.

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646.16

A CONNEXIN GENE HOMOLOG IN THE GIANT DANIO RETINA T.L.E. Wagner*, and D.G. McMahon. Department of Physiology, University of Kentucky, Lexington, KY 40536.

Gap junctions are closely apposed areas of cell membranes which contain gap junctional channels, or connexons. (Individual subunits are called connexins.) In the retina they mediate lateral inhibition in the outer plexiform layer by connecting horizontal cells. Retinal gap junctions also serve as an excellent model for gap junctions found throughout the nervous system.

We have cloned from the giant danio (*Danio aequipinnatus*) a partial cDNA (GDRET-1) which shares high sequence homology and similarly predicted structural features with previously cloned connexins. This clone was isolated from retinal RNA using reverse transcriptase polymerase chain reaction (RT-PCR) and a set of degenerate primers based on conserved areas of previously cloned connexins. The cloned sequence was compared to those of previously sequenced connexin genes in nucleic and amino acid forms (PCGENE, IntelliGenetics). Of the rat connexins previously cloned, GDRET-1 is most similar to rat cx43. Identity at the nucleotide level is 72%, and at the amino acid level is 73% with 79% homology. GDRET-1 has been confirmed as part of the giant danio genome by Southern analysis. Additionally, we have shown that GDRET-1 is expressed in the retina using RT-PCR with specific primers and Rnase protection assay.

We are presently creating giant danio retinal and brain cDNA libraries for use in isolating the full length clone. Supported by NIH EYO9256.

647.1

60-Hz MAGNETIC FIELD EXPOSURE REDUCES OCULAR PHOTOPIGMENT AND HEPATIC RETINOIDS IN *M. DOMESTICA*.

A.T.C.Tsin*, Y. Moreno, J.W. Rhodes, S. Ziari, M.de la Garza and N. Mata. Division of Life Sciences The University of Texas at San Antonio, San Antonio, TX 78249.

60-Hz magnetic fields (MFs) have been shown to affect neuroendocrine functions such as melatonin level in blood. However, it is not known if MFs might also influence visual function by a modification of visual pigment chromophore which is located in the hydrophobic pocket of the visual protein opsin. Adult female marsupials (*Monodelphis domestica*) were subjected to 60-Hz MFs for over 2 months prior to sampling. The MF, a circular rotating vector with rms flux density of 500 mG (50 uT), was on for 20 hrs out of each 24-hr day. Control animals were housed in the same building but located over 90 ft from the MF coils. Ambient 60-Hz MFs were 0.3 mG or less. No significant reduction in body weight and other visible pathology were noted in all animals. Retinas and livers were extracted for retinoids and analyzed by standard HPLC methods. All-trans and 11-cis retinal chromophores recovered from 60-Hz treated animals were reduced compared with controls (i.e. 44 vs 67 pmol/eye, all-trans retinal; 28 vs 35 pmol/eye, 11-cis retinal). Similarly, concentration of retinyl palmitate in liver of treated animals (0.5 mmol per gm liver) was lower than controls (2.4 mmol per gm liver). Liver retinol was also lowered in the treatment group (0.3 vs 1.32 mmol per gm liver in controls). The mechanism of action of MFs to reduce visual pigments and vitamin A in *M. domestica* is not known at present.

647.2

CHANGES IN THE PUPILLARY LIGHT REFLEX OF THE *RD* MOUSE WITH AGE AND THE EFFECTS OF PHOTORECEPTOR TRANSPLANTS

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The retinal degeneration (*rd*) mouse is a model for retinitis pigmentosa. It has a mutation in the β-subunit of cGMP-phosphodiesterase which causes photoreceptor degeneration starting at day 8 and complete by 3 weeks of age. The rapid and total loss of the photoreceptors renders the model particularly useful for investigating the potential of transplants to reconstruct the retina. Although a number of groups have transplanted photoreceptors to the subretinal space (Gouras et al., 1992; Invest. Ophthalmol. Vis. Sci. 33:2579-2586) there are no published data on the function of such transplants. Using a computerised pupillometry system we have studied the pupillary light reflex (PLR) of the *rd* mouse over time as a baseline for evaluating the function of retinal transplants in these animals. The amplitude, velocity and latency of the constriction was analysed at two different light intensities. The PLR was studied in *rd* mice at ages of 3 months, 6 months and 9 months. We found an increase in both the amplitude and latency of the PLR with time, at both light intensities. The response of 9 month old animals showed a greater amplitude and latency than the 3 month old *rd* mice.

Retinal dissociates from new-born mice were transplanted successfully into *rd* retinae. The anatomical evidence is presented. The responses recorded from eyes that had received transplants differed from those of control eyes.

Sponsored by Foundation for Fighting Blindness, and Cambridge Commonwealth Trust.

647.3

RETINAL DAMAGE OF SOLAR ORIGIN IN A VANISHING SPECIES OF AMPHIBIAN, *RANA CASCADAE*.

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Thinning of the stratospheric ozone layer is associated with a destructive effect of increased UV-B on egg-hatching success in *R. cascadae*, one of 5 Northwestern amphibian species undergoing severe population declines (Blaustein, et. al, PNAS, 1994).

Quantitative histopathological analysis of the outer retina in three groups of frog--adult *R. cascadae*, normal *R. pipiens* (northeastern leopard frog) and experimentally light-damaged *R. pipiens* -- was conducted. Both retinas from 3 frogs in each group were evaluated using samples taken from superior and inferior retinal regions, embedded in epon, sectioned at 1μ thickness and stained with thionin for light-microscopy evaluation.

Results: A similar profile of histopathological features were observed both in *R. cascadae* and in experimentally light-damaged *R. pipiens*: abnormal clumps of melanin pigment, abnormal rod outer segments, reduced number of cones, and vacuolated inner segments. In *R. cascadae*, the inferior retina was substantially more abnormal than the superior retina. Relatively few abnormalities were observed in normal *R. pipiens*. These findings are consistent with the hypothesis that increased UV-B in solar radiation may be having deleterious effects in retina of amphibians and contributing to the worldwide "vanishing amphibian" crisis. (supported by NSF Grant # IBN 921157 to K.V.F.)

647.4

FUNCTIONAL AND PATHOLOGIC CONSEQUENCES OF THE Q-64-TER RHODOPSIN MUTATION IN AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA.

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Clinical and histopathjologic observations were made on the retinas of a 50 year old patient donor with autosomal dominant retinitis pigmentosa due to the Q-64-ter stop codon rhodopsin mutation. The donor was a member of a large kindred whose affected members show variable severity of retinal disease. On clinical examination 4 mos *ante mortem*, the donor's acuities were 20/60, and kinetic perimetry showed a midperipheral scotoma with retained function centrally and in the far periphery. Test results in a mildly affected family member were consistent with reduction of rod outer segment membrane and existence of only wild-type rhodopsin in functioning rod outer segments. Microscopy of the donor retina revealed that rods and cones were reduced in number in the macula and far periphery and their outer segments were quite short. Rhodopsin was delocalized to the rod inner segments and somata, and many peripheral rods gave rise to rhodopsin-positive neurites that bypassed the inner nuclear layer and reached the inner limiting membrane. The rod neurites contained numerous multivesicular bodies and 50 nm vesicles that were synaptophysin and SV2 immunopositive. The delocalized rhodopsin in the rod somata and neurites was labeled with mAbs specific for the N and C termini of rhodopsin, indicating that at least some of it was wild type. Quantitative EM immunolabeling with the two anti-rhodopsin mAbs indicated that wild type but not mutant rhodopsin was present in the rod outer segments. The microscopic findings correlated well with results from visual function testing. Studies are in progress to determine if the rod neurite sprouting found in this and other RP retinas is a response to growth factor upregulation in response to ongoing photoreceptor death. *Supported by EY0-1311, -1730, & -5627, The Foundation Fighting Blindness, and Research to Prevent Blindness, Inc.*

647.5

FELINE RETINAL DEGENERATION RESULTING FROM DIETARY ß-ALANINE.

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It has long been known that cats are dependent on a dietary source of taurine to maintain their body pools because they have a limited capacity for biosynthesis. In this study we have used ß-alanine, which utilizes the same uptake system as taurine, in an attempt to lower taurine concentrations. Adult female cats were fed a completely defined taurine-free synthetic diet alone or supplemented with 0.05% taurine for at least 2 years and then provided with 5% ß-alanine in the drinking water for 20 weeks, each cat consuming approximately 500g ß-alanine. As noted in other species, ß-alanine reduced taurine concentrations globally in all cats.

Light and electron microscopic examinations of retinas from these cats revealed that ß-alanine ingestion accelerates the photoreceptor degeneration and elimination demonstrated previously in cats after a long period of taurine deprivation. The degree and extent of degeneration resulting from ß-alanine treatment was approximately proportional to the reduction in retinal taurine concentration, determined in the contralateral eye. Thus some cats fed the taurine-supplemented diet showed relatively normal retinal structure and the highest retinal taurine, others had reduced number of photoreceptors with markedly shortened or disorganized inner and outer segments and lower retinal taurine. Among the cats fed the taurine-free diets, one showed no remaining photoreceptors in any areas examined, and the lowest taurine in the retina, while others displayed varying degrees of degeneration. In general, the most severe changes were noted in the nasal and optic disc regions, and the least in the inferior region.

647.6

INCREASED EXPRESSION OF RETINAL TIMP3 mRNA IN SIMPLEX RETINITIS PIGMENTOSA IS LOCALIZED TO PHOTORECEPTOR-RETAINING REGIONS.

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The tissue inhibitor of metalloproteinases-3 (TIMP3) gene is the most recently characterized member of a family of genes whose products are implicated in extracellular matrix remodelling. We have shown by differential cDNA library screening and RNA-blot analysis that increased expression of TIMP3 mRNA occurs in retinas affected by simplex retinitis pigmentosa (RP). We here report the cellular distribution of TIMP3 overexpression in dystrophic retinas determined by *in situ* hybridization.

Paraformaldehyde-fixed control and RP-affected human retinas were frozen in isopentane and 10 μm sections cut on a cryostat. Acetylated sections were hybridized with ^{35}S-labelled sense and antisense riboprobes complementary to human TIMP3 and autoradiographed.

In control retinas, no TIMP3 mRNA expression was detected. In contrast, in RP simplex-affected retinas, a striking pattern of expression was observed. In regions lacking photoreceptors, no signal was detected. However, where there were residual photoreceptors, expression of TIMP3 mRNA was localized particularly to the photoreceptor inner segments and the inner retina.

The induction of TIMP3 mRNA overexpression in pre-degenerative photoreceptors suggests disruption of photoreceptor cell-matrix interactions, which in turn may lead to activation of apoptotic cell death programs.

Supported by grants from the British Retinitis Pigmentosa Society.

647.7

A CHANGE IN THE NUMBER OF NADPH DIAPHORASE-STAINED RETINAL CELLS IN STREPTOZOTOCIN-INDUCED DIABETIC RAT RETINA. E.Roufail[1,2], S.M.Rees[1], R.Rajan[3*] & D.Ehrlich[2]. [1]Department of Anatomy and Cell Biology, University of Melbourne, Parkville 3052,; [2]Department of Ophthalmology, University of Melbourne, Parkville, Victoria, 3052; [3]Department of Physiology, Monash University, Clayton, Victoria, 3186 Australia.

NADPH diaphorase (NADPHd) is a nitric oxide synthase (NOS) which is found in amacrine cells closely associated with the retinal vasculature in the rat retina. NOS is required for the production of nitric oxide (NO) a potent vasodilator and neuronal NOS may play a role in linking the control of the retinal vasculature with neuronal metabolism. We aimed to assess any changes that might occur in this population of neurons over time in streptozotocin (STZ)-induced diabetic rats where the retinal vasculature is known to be dysfunctional. Diabetes was induced with a single intravenous injection of STZ (70 mg/Kg) and rats allowed to survive for 3 weeks (n=9), 8 weeks (n=6) and 8 months (n=7).Treated and age-matched control rats were killed with an overdose of sodium pentobarbital and the right eye from each animal dissected and fixed in buffered 4% paraformaldehyde for 2h. The retina was flat mounted and stained for NADPHd histochemistry. Counting of stained-cells was performed double blind. By 3 weeks there was a significant decrease of 21% in the total number of positively-stained cells in the retinas of the diabetic group (2479±129) (mean±SEM) compared to the control group (3148±158) ($p<0.01$).The difference persisted at 8 weeks and 8 months. The observed decrease in NADPHd-positive cells in the diabetic rat retina suggests a possible loss of NOS activity and NO production which develops during the early stages of diabetes and persists long term. This loss of NOS activity may be involved in the early capillary changes which occur in the pathogenesis of diabetic retinopathy.

647.8

A NON-HUMAN PRIMATE MODEL OF RETINITIS PIGMENTOSA
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Our **purpose is** to develop and evaluate a primate model for human retinitis pigmentosa (RP), using sodium iodoacetate (IAA) to induce photoreceptor degeneration. Noell (1953) found that IAA induces a degeneration histologically similar to RP. We examined the ERG, fundus appearance and retinal histology in two macaque monkeys, which were followed for 16 and 19 months after acute systemic IAA administration. Visually guided behavior remained, and the effects in the two eyes were similar. A novel non-invasive quasi-focal cone ERG method (Wolf & Grosof, 1995) indicated that IAA spares the function of macular cones only and that the foveal cones were less affected by IAA than parafoveal cones. Over the course of several months cone ERGs decreased, and the fundus showed typical RP signs: vascular narrowing, waxy pallor of the disc, and retinal pigment epithelial cell and choriocapillary atrophy. Confirming Noell, we found the outer nuclear layer (ONL) to be devastated except in the central retina, where a gradient of damage extended from a relatively normal fovea to a monolayer of cone-like ONL somata outside the macula. The inner retina was grossly normal. Also observed in all the IAA retinas was the occasional RP complication of cystoid macular edema.

Conclusions: IAA acutely ablates the photoreceptor layer except in the macula. Macular cones surviving the initial toxic insult survive and function for many months, even as the retina appears to degenerate in an RP-like way. IAA treated primates show anatomical and physiological changes characteristic of RP, and therefore appear to be a useful model of RP.

Noell, WK *Am J Ophthalmol*, **36**: 103-116 (1953).
Wolf, MJ & Grosof, DH ISCEV Meeting, June, 1995.

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VISUAL CORTEX: STRIATE VI

648.1

REVERSE CORRELATION MAPPING OF SUBTHRESHOLD SYNAPTIC POTENTIALS IN CAT PRIMARY VISUAL CORTEX. L. Glaeser, V. Bringuier, Y. Frégnac*, L. Borg-Graham, C. Monier (1), and G. Fleury (2). Institut Alfred Fessard CNRS (1), and Ecole Supérieure d'Electricité (2), France.

Extracellular analysis of cortical receptive fields (RFs) have revealed the existence of "unresponsive" surround regions by their modulatory effect on the cell's response to a stimulus presented within the minimal discharge field (MDF). Obtaining direct evidence for subthreshold excitatory and inhibitory regions extending beyond the classical RF requires methods for generating, recording, and detecting physiologically identified EPSPs and IPSPs.

Simple cells in area 17 of anaesthetized cats were recorded using sharp intracellular and whole-cell patch electrodes while providing pseudo white noise input (small light and dark bars flashed randomly in the visual field every 50ms). The following algorithms were used to detect occurrence times of PSPs in the waveform: (a) threshold-crossing of membrane potential, (b) threshold-crossing of waveform energy calculated with respect to the average baseline within a brief time window, and (c) prototypical and waveform-specific template matching to discriminate between fast and slow IPSPs and EPSPs, Ca^{++} and Na^{+} spikes. Discrete event trains were correlated with the stimulus ensemble to yield high spatial resolution IPSP and EPSP maps.

Subthreshold maps show both excitatory and inhibitory regions extending up to nine times the area of the classical MDF. Map variance peaked when template matching was used (especially for inhibitory-like events). All excitatory profiles were consistent with stimulus-locked waveforms averaged at each spatial location for both types of stimuli. Moreover, the reverse correlation technique revealed inhibitory regions which were undetected using stimulus-locked averaging. The physiological nature of the subthreshold events detected was established from recordings at different membrane potentials and/or during QX 314 application.

We thank Ralph Freeman for providing stimulus generation algorithms. This work was supported by grants to Y.F. from HFSP and Conseil de l'Essonne.

648.2

ROLE OF VOLTAGE DEPENDENT INACTIVATING CONDUCTANCES IN THE CONTROL OF THE VISUAL INTEGRATION FIELD PROFILE IN CAT AREA 17
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Neurons of the primary visual cortex integrate information over a visual area larger than their minimal discharge field (MDF). Intracellular recordings in cat area 17 *in vivo* allowed us to study responses to peripheral stimuli, their voltage and time-dependency.

Our stimuli consisted of gratings drifting for 400 ms in a restricted portion of the visual field. Four types of spatial configurations were used: inside the MDF, "near" periphery, "far" periphery and "global" ("near"+"far") periphery. Voltage dependency was studied by coupling different levels of depolarizing (usually subthreshold) and hyperpolarizing current steps with visual activation. Temporal dependency was assessed by setting the onset of the current pulse either 1000 ms before or coincident with the initiation of the visual response.

In all cells studied, peripheral stimulation from regions up to 10° away from the border of the MDF elicited a response. At resting potential, these responses were usually depolarizing, but hyperpolarizations were elicited in a few cells. Some hypercomplex cells showed a pure *excitatory* response to a stimuli localized in the end-zones and the side bands. When weak or absent at rest, visual depolarizing potentials could be enhanced or revealed by current-induced *depolarizations*. Finally, temporal coincidence of the current step and visual response could further boost excitatory potentials with respect to the one second delayed response. We hypothesize that voltage dependent inactivating conductances in the vicinity of the post-synaptic site may regulate the gain of these connections.

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648.3

THE CONTRIBUTION OF LGN M AND P LAYERS TO THE CONTRAST SENSITIVITY OF PRIMATE V1 NEURONS. **J.D. Allison[1] *, P. Melzer[1,2], Y. Ding[1], J. Dinger[2], A.B. Bonds[3], and V.A. Casagrande[1,2]. Depts. of Cell Biology[1], Psychology[2] and Electrical Engineering[3], Vanderbilt University, Nashville, TN 37232.**

Magnocellular (M) neurons in the LGN of primates, including bush babies, exhibit higher contrast sensitivity (i.e. lower contrast thresholds) and saturate at lower stimulus contrast than parvocellular (P) LGN neurons. The contributions of M and P LGN neurons to the contrast sensitivity of individual V1 cells are unknown. To examine this issue we inserted an injection/recording electrode containing 25 mM GABA in saline into either the contralaterally innervated M (layer 1) or P (layer 6) layers of the LGN in 8 bush babies. Multiunit activity was recorded to identify the layers and locate the receptive fields. We then inserted a recording electrode into V1 and, after lining up the cortical and LGN receptive fields, measured the contrast response functions (CRFs) of single V1 neurons using spatio-temporally optimized drifting sine-wave gratings presented to the contralateral eye. The CRF of each V1 neuron was retested after blocking activity in either the M or P layer with an injection of GABA. When the M layer was blocked ($n=6$), the average contrast sensitivity of V1 neurons was reduced from 16.8 to 8.2 ($t=2.95$; $P<0.05$). When the P layer was blocked ($n=6$), the average peak response amplitude at high stimulus contrast levels (i.e. 56%) was significantly reduced from 21.4 spikes/s to 8.3 spikes/s ($t=1.95$; $P<0.05$). M cells contribute predominantly to the low contrast/high sensitivity component of the CRF of V1 neurons while P cells mainly contribute to the high contrast/low sensitivity component. These results suggest that V1 neurons receive input from *both* M and P LGN cells.

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648.4

TEMPORAL PROPERTIES OF MAGNO AND PARVO SUBSYSTEM INTERACTIONS: VEPS TO SIMULTANEOUS OR ALTERNATING STIMULATION. S. Suter*, P.S. Suter, D.T. Perrier, B.J. Gragg and T.B. DeLouth. Vision Laboratory, Psychology Dept., California State University, Bakersfield, CA 93311.

The magnocellular (M) inhibits the parvocellular (P) visual subsystem. We present VEP evidence that M/P interactions are subject to important temporal influences. Steady-state VEPs (2nd harmonic) were recorded (Oz to T3) in two experiments from 21 and 22 adults. Stimuli subtending 14 deg were horizontal gratings differing in spatial and temporal frequency and contrast (M = 0.86 c/deg with 17%C at 15 Hz, P = 5.00 c/deg with 84%C at 3 Hz). In Exp. 1, M and P stimuli were rapidly alternated. P duration was always 1.667 s; M duration varied across trials from 10.667 to .333 s. M amplitudes increased by 35% and P amplitudes decreased by 65% as M duration shortened. These results are consistent with either preferential adaptation of P across time, or optimal P inhibition during shorter M durations. In Exp. 2, M and P stimuli were presented for 45-s trials either simultaneously by interleaving video frames, or separately. For both conditions, M amplitudes did not increase, whereas P amplitudes increased across the first 20 s. Thus, the decreased P amplitudes in Exp. 1 are not due to P adaptation. Consistent with M on P inhibition, P amplitudes were smaller in the simultaneous condition, while M amplitudes did not differ between conditions. The M subsystem achieves steady-state quicker as compared to the P subsystem. M on P inhibition is greatest when M is stimulated for brief intervals.

648.5

EFFECTS OF ADAPTING ON SPATIOTEMPORAL RECEPTIVE FIELD STRUCTURE IN CAT STRIATE CORTICAL SIMPLE CELLS. A.B. Saul*. Dept. of Neurobiology, U. of Pgh. Sch. of Med., Pittsburgh PA 15261.

Adapting changes timing, as well as amplitude (Vis. Neurosci. 12:191-205, 1995). Simple cell responses to drifting gratings are delayed at onset, but unaffected at offset. By examining receptive field structure in control and adapted states, we have gained some insights into the origins of these specific aftereffects. These studies also address general issues of the generation of response properties.

Simple cells were recorded in anesthetized, paralyzed cats. Maps were obtained using both sinusoidally-modulated and briefly flashed bars. The 4-second trials that generated these maps were preceded by 8-second periods in which the screen was either blank, to obtain control maps, or in which a drifting grating was presented to obtain adapted maps, with all conditions interleaved. Analysis of the responses consisted of amplitude and timing measures at each position, and predictions of responses to drifting gratings.

Both amplitude and timing of responses to stationary stimuli were altered by adapting. The main result is that the predictions of the drifting grating responses from these stationary maps showed the characteristic delay of response onset after adapting. Although these predictions integrate responses at all receptive field positions, the effects were generally due to delayed onsets at just a few positions. Predictions of the aftereffects in spatial and temporal frequency tuning also corresponded with results obtained from drifting gratings.

Previous studies have demonstrated through population comparisons that receptive field structure is correlated with response properties. The current experiments extend these results to comparisons within single cells. They also extend our notions of how specific adaptation aftereffects are, showing differential behavior in different parts of the receptive field. These new data are consistent with a model where adapting potentiates inhibition between simple cells that are in push-pull arrangements.

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648.6

STROBE REARING ALTERS THE SPATIOTEMPORAL STRUCTURE OF SIMPLE CELL RECEPTIVE FIELDS IN CAT AREA 17.

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In most simple cells, a link exists between spatiotemporal (S-T) receptive field structure and direction selectivity (DS). S-T oriented receptive fields possess gradients of response timing which confer a preferred direction of stimulus movement across the receptive field. Non-DS cells lack S-T orientation. Here we further examine the link between receptive field structure and DS using a developmental paradigm that specifically affects direction selectivity.

Cats were raised from birth to 8 mo. of age in 8 Hz strobe illumination. Drifting gratings were used to measure the selectivity of >40 cells for stimulus direction, spatial and temporal frequency and contour orientation. Receptive field structure was assessed using stationary, sinusoidally modulated bars, which allowed measures of response latency and absolute phase at different positions in the receptive field.

Cortical cells were normal in all measures except DS and S-T structure. Fewer than 10% of the cells were DS, compared to >80% in normally reared cats. All non-DS cells lacked S-T oriented receptive fields; they displayed uniform response timing within each ON and OFF zone and abrupt jumps in timing between zones, like non-DS cells in normally reared cats. In strobe cats, this change in S-T structure is due in part to an absence of receptive field positions with absolute phase lags and long latencies. Such positions are observed in normally reared cats and may reflect inputs from lagged cells of the lateral geniculate nucleus. Their absence in strobe cats may indicate failure of the lagged afferents to drive cortical cells.

In summary, these results provide further support for the role of S-T structure in conferring direction selectivity in simple cells. Strobe rearing eliminates direction selectivity by altering the timing of cortical responses within the receptive field.

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648.7

A ROLE FOR $GABA_a$-MEDIATED INHIBITION IN GENERATING THE SPATIOTEMPORAL STRUCTURE OF SIMPLE CELL RECEPTIVE FIELDS.

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Many simple cells possess gradients of response timing across their receptive fields. This property is known as spatiotemporal (S-T) orientation, and it is correlated with cells' direction selectivity (DS). The mechanisms underlying S-T orientation are unknown; they may reflect inputs from the lateral geniculate nucleus, the striate cortex, or both. Here we examine the role of intracortical inhibition.

The $GABA_a$ antagonist N-methyl-bicuculline (NMB) was iontophoresed onto simple cells in cat area 17, and its impact on S-T structure and DS were measured. S-T structure was determined using stationary, sinusoidally modulated sinewave gratings at different spatial phases. Direction selectivity was measured with drifting gratings. All stimuli were randomly interleaved in the control and NMB conditions. The first harmonic response was a valid measure, even during NMB application.

Of 11 direction-selective cells with S-T oriented receptive fields, 7 showed NMB-induced reductions in DS and S-T orientation. Response timing became more uniform across their receptive fields. For these cells, changes in the two measures were correlated ($r=0.87$; $p<0.01$). In contrast, the other 4 direction-selective cells showed little or no change in DS despite NMB-induced increases in their visually driven activity. S-T orientation in these cells was affected more variably.

These data show that S-T structure and DS covary in single cells; this extends previous observations based on comparisons among populations of cells. In addition, the data reveal that $GABA_a$-mediated inhibition can act to create direction selectivity through its impact on S-T structure. However, heterogeneity appears to exist among simple cells in the dependence of their direction selectivity on this inhibition.

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648.8

SPATIAL SUMMATION PROPERTIES OF THE MACAQUE V1 NEURONS IN THE RETINOTOPIC REPRESENTATION OF THE BLIND SPOT.

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In a previous study (*Neuroscience Research Suppl.* 19), we have shown that when a large stimulus covering the blind spot (BS) is presented monocularly, it activated about 15% of neurons in the retinotopic map of the V1 representing the BS. Many of these neurons were located in layer 6, and most of them had very large receptive fields that covered large parts of the BS and extended out of it. We suggested that these neurons may consist of an important part of the neural mechanisms of filling-in at the BS by importing visual information from the surround to the inside of the BS in the retinotopic representation in V1. In the present study, we examined the spatial summation properties of these neurons in order to determine the spatial characteristics of such huge receptive fields. Two monkeys were trained a visual fixation task, and visual responses of neurons were recorded using stationary stimuli of various sizes and at various locations with respect to the BS. The following two properties were found. (1) Many of these neurons preferred larger stimuli to some extent, in contrast to most of the V1 neurons which are suppressed by a large stimulus. This suggests that spatial summation of visual information takes place across a rather large visual field. (2) In some of the neurons, onset of the response was delayed systematically with increasing the distance between the stimulus and the center of the receptive field, whereas the magnitude of the responses was not decreased systematically. This seems to suggest that propagation of activities involved in the formation of a huge receptive field occurs in a cortical level.

648.9

EVIDENCE FOR SCALE-INVARIANT MEDIAL AXIS REPRESENTATION IN PRIMATE STRIATE CORTEX.

T.S. Lee*, Dept. of Brain and Cognitive Sciences, MIT; Div. of Applied Sciences, Harvard University.

Striate cortical complex cells' responses are enhanced preferentially at the boundaries and at the center of texture squares and texture strips [Lee et al. ARVO 1995]. We hypothesized that the response peak close to the center of the texture figure is the neural correlate of the medial or symmetry axis representation of a visual object. To test this hypothesis, we isolated 43 complex cells in the striate cortex of an awake behaving rhesus monkey and studied their responses to texture strips of different widths, ranging from 2 degrees to 6 degrees in visual angle. The cells' RF were located at $3° \pm 0.7°$ eccentricity from the fovea. Each strip stimulus was presented at a randomized series of sampling positions relative to the cells' CRF (size $< 0.3°$) while the monkey fixated. The spatial sampling intervals were 0.5° for the 6° and 4° wide strips, 0.375° for the 3° wide strips, and 0.25° for the 2° wide strip. 9 of the 25 cells that were tested with 6° strips, 23 of the 37 cells tested with 4° strips, 8 of the 13 cells tested with 3° strips and 4 of the 4 cells tested with 2° strips showed an enhanced response at the center of the strips. 10 of the 28 cells that were examined for at least two widths exhibited central response peaks across scale. Strips were also sampled across different cross-sections and the enhanced responses were observed along the midline of the strips. This invariance of response enhancement along the midline and across multiple widths further suggests the plausibility of a medial axis representation in V1. The decrease in the proportion of the cells exhibiting the medial peaks with increasing strip width reflects a spatial constraint that limits the medial axis computation to a narrow range of spatial scale at each eccentricity. However, strips of comparable widths elicit stronger medial response at 5° eccentricity, indicating that the medial axis of different ranges of object dimension might be computed at different eccentricities in V1.

648.10

DO SIMPLE CELLS IN THE CAT'S STRIATE CORTEX ENCODE BINOCULAR DISPARITY THROUGH POSITION AND PHASE INCONGRUITIES?

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The visual system utilizes binocular disparity to obtain information about the relative depth of objects in space. Most cells in the striate cortex are selective to binocular disparity, suggesting that these neurons form the first processing stage for stereopsis (Barlow et al. 1967; Pettigrew et al. 1968). It has been suggested that binocular disparity is encoded by spatial offsets (position disparities) of left and right eye receptive fields (RFs) that have identical internal structure (Nikara et al. 1968; Maske et al. 1984). However, we have shown with detailed RF mapping studies that RF profiles for the two eyes are not necessarily the same and that binocular disparity can be encoded through differences in RF phase between the two eyes without position disparity (Freeman & Ohzawa, 1990; DeAngelis et al. 1991). This raises a question as to whether simple cells encode binocular disparity through phase disparity alone or through both position and phase disparities. To answer this question, we simultaneously map RFs of multiple simple cells in anesthetized and paralyzed cats to obtain phase disparity for each cell as well as position disparity between the cells. We then determine the relative contributions of phase and position disparities to the binocular disparity tuning of the cells.

Dynamic noise patterns generated according to binary m-sequences are presented over the RF locations on two graphic displays, one for each eye, to map left and right eye RFs simultaneously. Responses of cells to the noise patterns are cross-correlated with the stimulus sequence to obtain RF maps. These maps are then fitted with a Gabor function to estimate phase and center coordinates of the RFs. Phase and relative position disparities are obtained from the estimated parameters.

We find that the majority of simple cells exhibit incongruities in position as well as in phase. Although the contribution of position disparity to the disparity preference of some cells is substantial, position disparity is generally limited to the extent that is equivalent to a 90° phase disparity. Therefore, we conclude that these simple cells encode binocular disparity through both position and phase incongruities but position disparity alone may not be sufficient to encode large disparities. (EY01175)

648.11

COMPARISON OF THE CLASSICAL AND THE INTEROCULAR SUPPRESSIVE RECEPTIVE FIELD USING RECEIVER OPERATOR CHARACTERISTIC ANALYSIS REVEALS A CLOSE SPATIAL CORRESPONDENCE IN ANAESTHETISED CAT. Tobe C.B. Freeman, Frank Sengpiel, and Colin Blakemore*. University Laboratory of Physiology, Oxford OX1 3PT, U.K.

Interocular suppression is observed in cat primary visual cortex when a cell responding to an optimal grating in one eye is presented with an orthogonally oriented stimulus to the receptive field in the other eye (Sengpiel et al. 1995. Vision Research 35:179-95). We sought to determine the area within the visual field over which this suppressive binocular interaction could be evoked. The entire receptive field of the dominant eye was stimulated with a drfiting sinusoidal grating of optimal orientation and direction of drift. The receptive field of the other eye was probed with a small, orthogonally oriented drifting grating patch placed in random sequence in a matrix of positions within and around the classical receptive field. Using a monocular probe stimulus of optimal orientation, the classical receptive field in that eye was also mapped in a similar fashion.

We used Receiver Operator Characteristic (ROC) analysis to estimate the significance of the changes in firing rates. The technique allowed us to make direct statistical comparison between the classical and suppressive receptive field profiles in the same eye.

We find that the spatial organization of the interocular suppression field matches closely that of the classical receptive field in both location and size (70 cells, of which 15 were analysed in detail). Interocular suppression can be elicited over a range of non-optimal orientations and spatial frequencies (Sengpiel et al. op. cit.), while its spatial extent is similar to that of the classical receptive field.

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648.12

TWO DIFFERENT MECHANISMS UNDERLIE SUPPRESSIVE PHENOMENA IN CAT PRIMARY VISUAL CORTEX. Frank Sengpiel*, Roland J. Baddeley, Tobe C.B. Freeman, Richard Harrad[1] and Colin Blakemore. University Laboratory of Physiology, Oxford OX1 3PT, U.K. and [1]Bristol Eye Hospital, Bristol BS1 2LX, U.K.

In cat area 17 (V1) the response of a cell to an optimal stimulus in one eye can usually be reduced by an orthogonally oriented grating either presented to the other eye (*interocular suppression*) or superimposed in the same eye (*cross-orientation inhibition*). Sengpiel et al. (1994) reported that interocular suppression causes a reduction in response proportional to the unsuppressed level of response, with no shift of contrast threshold. But Bonds (1989) reported that cross-orientation inhibition raises contrast threshold without changing response *vs* contrast gain. Here we compared these two types of suppression, and *surround inhibition*, in the same neurons.

For 25 neurons in V1 of anaesthetized paralysed cats, we measured response, R, to 1) dichoptically presented orthogonal gratings, 2) superimposed orthogonal gratings, and 3) a circular grating patch surrounded by an iso-oriented grating in an annular window (so as to elicit surround or end inhibition). In each condition, the contrast c of the excitatory stimulus was varied randomly, while that of the suppressive grating was kept constant. Data were fitted by a hyperbolic ratio function, $R = R_{\max} \cdot c^n / (c_{50}^n + c^n) + b$.

For all cells tested, cross-orientation inhibition was best described as an increase in c_{50}, while for 53% of cells, interocular suppression was best described as a reduction in R_{max}. Inhibition by surround stimuli was about equally well fitted by changes in either c_{50} or R_{max}. Our data suggest that different inhibitory mechanisms, one 'divisive' and one 'subtractive', may operate in V1 to mediate the various types of suppression.

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648.13

SPATIAL PHASE GRADIENTS IN NEOCORTICAL EEGs GIVE MODAL DIAMETER OF "BINDING" DOMAINS IN PERCEPTION. W. J. Freeman*, J. M. Barrie, M. Lenhart, R. X. Tang. Dep't of Molecular & Cell Biology, Univ. of California, Berkeley CA 94720

Spatial patterns of EEGs were derived from 8x8 epidural electrode arrays (0.5-0.8 mm spacing) on the olfactory bulb (OB), prepyriform cortex (PC), and the visual, auditory and somatic cortices of rabbits. Phase was measured for components of the aperiodic wave forms in segments (64-512 msec) with AM-FM modulated Fourier components. Phase gradients in the OB had the form of a cone, for which the location and signs of the apices (maximum lead/lag) varied randomly. Phase velocity converged to 1.80±0.43 in m/s, the estimated conduction velocity of mitral-tufted axon collaterals. Most (80%) phase gradients in PC had the conduction velocity of olfactory tract (2.6 m/s). Others were endogenous gradients at 1.0 m/s for activity in low gamma and high beta ranges (20-60 Hz). Neocortical velocities had skewed distributions, with modes dependent on frequency, from 2.0 m/s at 22 Hz to 3.5 m/s at 48 Hz. Commonality ("phase locking", "binding") was defined as oscillation within the half power range (± cos 45º). An invariant estimate emerged on multiplying the phase velocity in mm/ms by 0.25 cycle in ms = 21.0±0.5 mm for modal diameter of most neocortical domains, but 6.1±2.5 m/s for some in somatic cortex. This is consistent with ECoG data from exposed human cortex (<2 cm) and with cat unit recordings (for example, at 40 Hz the estimated lag across 7 mm is O.55 ms).

648.14

INTER-OCULAR DISPARITY AND VARIATION IN RECEPTIVE FIELD POSITION AND ORIENTATION PREFERENCE STUDIED BY TETRODE RECORDINGS IN CAT AREA 17. P. A. Hetherington*, N. V. Swindale, & D. Zhao. Department of Ophthalmology, University of British Columbia, 2550 Willow St., Vancouver, BC, Canada, V5Z 3N9.

Tetrodes are four closely-spaced metal microelectrodes that allow the simultaneous recording of multiple single units. We used the tetrode technique to study variations in receptive field position (RFP), orientation preference (OP), and inter-ocular orientation and receptive field disparity among local (100µm) groups of neurons in cat area 17. Units were stimulated with moving bars (0.25-0.5° x 1.5-2.0°) and isolated off-line using the peaks, valleys, and phase angles of the spike waveforms from the 4 channels. Our preliminary observations are based on recordings from 77 cells in 15 sites in area 17 of paralyzed cats anesthetized with isoflurane and NO_2. Between 3 and 9 cells were simultaneously recorded at each site. In agreement with a previous study using tetrodes (Maldonado & Gray, 1994, *SNA*, 266.10), the average maximum range in OP between cells of the same cluster was 28.6°, and varied from 9.1° to 85.5°. The variation in OP of cells within a cluster receiving ipsilateral eye input was correlated (r=.77) with the variation in OP of the same cells receiving contralateral input. The average amount of variation in OP within an eye (ipsi=4.91°, contra=5.11°) was slightly greater than the average variation between the eyes (3.18°). The RFP also varied within clusters: the average maximum range in RFP between cells of the same cluster was 1.10°, and varied from 0.41° to 1.67°. The average RF width (s.d. of a best-fit Gaussian) was 0.59°, and varied on average 0.42° within a cluster. The RFP scatter was greater in the horizontal (0.36°) than in the vertical (0.28°) dimension, but this difference was not statistically significant (p=.09). Ipsilateral RFP variation was similar to the variation in contralateral RFP within a cluster (0.53° vs. 0.57°), and there was an average of 0.46° inter-ocular disparity in RFP (N=37). Because RFP and OP were measured simultaneously in neighboring cells, it is unlikely that the variations within each cluster were due to eye movements. These variations among neighboring neurons may be necessary for dimension reduction in cortex. Supported by grants from MRC Canada to NVS and from NSERC to PAH.

648.15

SYNCHRONIZATION OF SINGLE UNIT SPIKE TRAINS IN CAT VISUAL CORTEX IS STIMULUS DEPENDENT

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Theoretical considerations and results from multi-unit recordings suggest that synchronization of neuronal activity could serve as a binding mechanism in the formation of neuronal assemblies. To investigate, whether the synchronization between *single cells* depends on stimulus properties, we recorded simultaneously with several stereotrodes multiple single units in area 17 of the anaesthetized cat. Amplitude and position of the correlation peaks were strongly influenced by the orientation of the moving bar stimuli. The changes of correlation peak position followed in most cases the rule that the more strongly activated cell leads by up to 10 ms over the other. The effect of different global stimulus configurations on the synchronization between pairs of single cells was studied with two different paradigms. If the receptive fields (RFs) of both cells were spatially separated and colinearly oriented, they were simultaneously activated either by a long bar moving over both RFs or by two independent bars moving in opposite directions, each one over only one of the two RFs. Neurons with overlapping RFs but different preferred orientations were both activated either by a single stimulus, or with two bars, their orientations matching approximately the respective preferences of the two cells. We found in both paradigms that for cells which had synchronized their spikes in response to a single coherent stimulus synchronization was reduced or absent when they were activated by two incoherent stimuli. The reverse pattern was never observed.

We conclude that correlations between single units depend on the actual stimulus configuration and do not directly reflect the fixed anatomical connectivity. Furthermore, the results indicate that synchronous activity could serve to define the set of neurons whose responses represent features of the same visual stimulus.

648.16

INTERHEMISPHERIC SYNCHRONIZATION IN CAT VISUAL CORTEX IS COMPARABLE TO SYNCHRONY WITHIN HEMISPHERES. A.K. Engel*, P. König, P.R. Roelfsema, M.H.J. Munk and W. Singer. Max-Planck-Institut für Hirnforschung, Deutschordenstr. 46, 60528 Frankfurt, Germany

Recent studies of neuronal interactions in the visual cortex indicate that synchronous firing of neurons may be relevant for the integration of distributed responses into coherent representational patterns. One important step in validating this hypothesis is the demonstration that synchrony can also occur between neurons located in different cerebral hemisperes. Several years ago, we have reported the existence of such interactions in cat visual cortex. We have now investigated the features of interhemispheric synchronization in greater detail to facilitate comparison with the synchrony observed within each hemisphere. Multiunit and field potential responses were recorded simultaneously from left and right area 17. Our results demonstrate that strong temporal correlations with a precision in the millisecond range can be observed between neurons in left and and right striate cortex. On average, the synchronization occurred without phase lag. The strength of the interactions was comparable to that observed within each hemisphere and was dependent on the overlap of the receptive fields but not on differences in the neurons' preferred orientations. If synchronization occurred, it was almost always associated with an oscillatory modulation of the responses at frequencies in the range of 30-80 Hz. Similar to the synchrony observed within hemispheres, the interhemispheric interactions were influenced by the configuration of visual stimuli. Synchrony was weak for incoherent stimulus arrangements and was higher if stimuli with similar orientation or direction of motion were used to coactivate different cell groups. The interactions were completely abolished in cats where the corpus callosum had been sectioned prior to the physiological experiment, demonstrating that the synchronization is mediated by connections at the cortical level and not by driving common input from subcortical structures. Taken together, the data are compatible with the hypothesis that synchrony may be exploited to establish relationships between responses of spatially distributed neurons, synchronization across the hemispheres then serving for the binding of signals arriving from different visual hemifields.

(AKE acknowledges support by the Heisenberg Program of the DFG.)

648.17

NEURONS RESPONDING WITH DIFFERENT LATENCIES AND TIME COURSES TO A VISUAL STIMULUS ENGAGE IN NEAR-ZERO DELAY MUTUAL CORRELATION IN THE VISUAL CORTEX OF AWAKE MONKEY. R.Eckhorn*, R.Rutschmann, T.Woelbern, A.Frien, R.Bauer. Deptm. Physics, Philipps-University, Renthof 7, D-35032 Marburg, Germany.

We studied visual temporal coding by investigating the internally generated signal correlations of neurons activated by the same visual stimulus. Single- (SUA), multiple-unit (MUA), and local field potential (LFP, 10-140 Hz) were recorded in the striate cortex of an awake macaque monkey by a linear array of 7 fiber μ-electrodes and analyzed by cross-correlation methods. During a fixation task the RFs were stimulated by a sinusoidal grating (switched on at preferred orientation and spatial frequency, stationary for 1s, then drifting at a local temporal modulation of 2 Hz for 3s). Classification of neurons into simple and complex (classical test of spatio-temporal modulation) did not reveal the separable classes as in anesthetized animals. Nevertheless, we divided the distribution into simple- and complex-like cells according to their depth of modulation. Their response latencies to the onset of the grating were not different, having a broad temporal dispersion with groupings around 50, 100 and 145 ms (N=72). Activities were typically non-rhythmic before and during the rising phase of the transient response to stimulus onset. During later response epochs, including stationary and slow movement stimulation, fast oscillatory components of high amplitudes dominated the neural signals (35-60 Hz). Both, phasic and rhythmic response epochs revealed cross-correlation center peaks close to zero delay (few ms) including SUA-LFP, MUA-MUA and MUA-LFP correlations at the same and different recording sites (cortical distance: 0-4.5mm). The SUA-LFP correlation peak delay differences of stationary and moving gratings were significantly less than zero ($p<0.01$) and showed slightly different values for simple- and complex cells (C: -0.09±0.20 ms, N=18 / S: -0.34±0.32 ms, N=29). The present results support previous observations of cortical temporal coding around zero-delay. Near-zero correlation was present independent of onset response latency and PSTH-course, non-rhythmic and rhythmic epoch, small and large cortical separation, simple and complex cell, MUA and LFP. However, resolving cross-correlations over response time revealed rather complex temporal patterns depending on neuron and stimulation. (Supported by DFG: Ec53/6 and 53/7 to R.E.)

648.18

OSCILLATORY GROUP ACTIVITY REVEALS SHARPER ORIENTATION TUNING THAN CONVENTIONAL MEASURE IN PRIMARY VISUAL CORTEX OF AWAKE MONKEY. A.Frien, R.Eckhorn, T.Woelbern, R.Bauer, H.J. Reitboeck*. Deptm. Biophysics, Philipps-University, D-35032 Marburg, Germany

Orientation selectivity is a prominent characteristic of neurons in the visual cortex. We asked, whether fast oscillatory group signals (35-60Hz) show sharper orientation tuning than the conventional measure (integrated response). This is expected if neurons that code similar visual features (e.g. orientation) engage in mutually facilitating oscillations. From 7 fiber μ-electrodes we recorded single and multiple unit activity (MUA) and local field potentials (LFP, 10-140 Hz) in the macaque striate cortex. In a fixation task the RFs were stimulated by a sinusoidal grating at different orientations. Orientation tuning of MUA in V1, utilizing the conventional measure, was nearly as sharp as for single units (73.6±27.6°, N=70). The latter exceeds reported values [e.g., Vogels et al, Exp Brain Res, 84:1, 1991] which is mainly due to our sinc interpolation algorithm. In addition, we found that oscillatory components of MUA reveal a significant reduction in the halfwidth of the orientation tuning compared to that of the integrated response ($p < 0.01$; difference: 8.4±10.5° / oscill: 75.4±16.0° / integr: 83.8°±20.2°). For oscillatory MUA components 100 (of 119) recording positions were sharply tuned while there was not a single for oscillatory LFP ("sharp tuning": response drops below half of its maximal value). Therefore we calculated an index of orientation (OI = max. response / min. response - 1), obtaining also significantly sharper orientation tuning for LFP oscillations than for the integrated LFP response ($p < 0.05$; OI(oscill) = 0.40±0.28, OI(integr) = 0.30±0.17; N=119). The large width of the present LFP orientation tunings is in contrast to the sharp orientation tuning of previous results for stimulation with moving bars [Eckhorn et al, NeuroReport 4:243, 1993]. This effect is probably due to the different extent of the cortical circuits activated by the two stimuli. In summary, our results support previous proposals that neurons of similar orientation preference engage in a common, synchronized oscillation when stimulated close to this orientation. The sharper tuning of oscillations compared with the integrated responses proves, in addition, that the oscillatory components mutually facilitate while this is not the case for the other components. It is probable, therefore, that this facilitation has implications for the perception of oriented visual structures. (Supported by DFG Ec 53/6 and 53/7 to R.E.)

648.19

VISUALLY INDUCED GAMMA BAND RESPONSES IN HUMAN EEG

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On the basis of animal studies, gamma-band oscillations have been proposed as a possible fundamental physiological mechanism solving the binding problem. In contrast to evoked gamma-band responses (GBR), induced GBRs are neither time nor phase locked to the stimulus onset. Averaging of trials most certainly will result in the disappearance of GBRs in the grand mean. In addition, the anatomical location of Area 17 may lead to a neutralization of electrical dipoles when visual full field stimulation is carried out.

Seven subjects were either stimulated with a long moving bar (coherence) or two bars moving in opposite direction (incoherence) on the left side of the screen, while fixating a cross at the center. Electrodes were placed pario-occipitally (10-20 system). Single trials were analyzed by Gabor transformed spectral analysis. The resulting evolutionary spectrum showed significantly higher gamma band activity in combination with alpha suppression at the contralateral hemisphere during coherent movement as compared to incoherent movement.

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VISUAL CORTEX: STRIATE VII

649.1

MAXIMIZATION OF INFORMATION IS CONSISTENT WITH THE ORGANIZATION OF VISUAL CORTEX. B.J. Richmond* and G.X. JIN. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

The patterns of connections among neurons probably arise from genetically specified rules rather than from the exact specification of structure. We have studied the consequences of one simple rule: that the network has developed to preserve as much information as possible across neuronal layers (the biologically-inspired Hebb rule is a special case of this). We considered 4 structures for a layer of neurons, adapting each according to this rule. (1) The neurons have independent noise on the outputs and noiseless inputs (a case examined closely by Linsker). (2) The neurons have correlated noise on the outputs and noiseless inputs. (3) The neurons have correlated inputs with no further noise added. (4) There is correlated noise on the inputs and the neurons have correlated instrinsic noise which is independent of the noise on the inputs. These different models make different predictions about the correlation of noise on the inputs with the correlation of signals on the outputs, and the role of noise in the speed of network adaptation. We compared the first prediction, i.e., the effect of correlated input noise on correlated output signal, with recordings of neuronal pairs made by Gawne and Richmond in V1 and IT cortices, and Ghose and Freeman in V1. For Gawne and Richmond, the input noise was correlated at about 5% variance, and the output information was about 10% redundant. Ghose and Freeman found the same input noise correlation and about 15% signal correlation. The model with correlations on both the inputs and within the layer (model 4 above) matches the experimental results almost exactly. The results are consistent with the assumption that the visual cortex self-organized according to a rule that maximizes the preservation of local information across layers.

649.2

TIME SCALES IN THE INFORMATION PROCESSING DYNAMICS OF PRIMATE STRIATE CORTICAL COMPLEX CELLS. G.X.Jin* ,T.J.Gawne, J.A.Hertz, and B.J.Richmond. Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda MD 20892, and NORDITA, DK-2100 Copenhagen, Denmark.

The time scales associated with the dynamics of neural information processing are not well understood. We measured information transmission in the responses of V1 complex neurons in awake monkeys to sequences of two-dimensional black-and-white patterns presented as a movie in both fixed and random orders. The speed of the movie was varied, the duration of each pattern in the sequence ranging from 16ms to 128ms.

The responses elicited by the fixed sequence were quite sterotyped, with pulses related to the pattern change often appearing to be aligned across trials, as reported by Bair and Koch for MT neurons. For the random sequences, we estimated the information transmitted about stimulus pattern using a neural network. We found: (1) The stimulus-related information rises as the duration of the patterns in the sequence increases. (2) Information begins rising about 60ms after the stimulus appears. (3) Information rises steeply during the next 40ms and then continues to rise more gradually through at least another 90ms. (4) The duration of the information rise is never longer than the length of the presentation period for a pattern. (5) The rate of information transmission peaks between 10 and 20 bits/sec, and remains high for a substantial proportion of the stimulus duration. Points (3) and (5) taken together show that the response carries a large amount of redundant information. (6) There is information about the preceding stimulus in the visual response to the current stimulus, i.e., there is a short memory of the preceding stimulus; this memory lasts for at least 30 ms.

649.3

HOW MUCH INFORMATION IS CARRIED BY CORRELATED NEURONS? P.E. Latham*, G.X. Jin, T.J. Gawne, and B.J. Richmond. Lab. of Developmental Neurobiology, NICHD, and Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

Experimental results from V1, IT and MT indicate that neuronal responses are correlated ($\rho_S \equiv$ signal correlation $\approx 15\%$ and $\rho_N \equiv$ noise correlation $\approx 5\%$). How much do these correlations affect the amount of information a population of M neurons can carry about a stimulus? For a Gaussian signal and noise, and a signal to noise ratio of S/N, the mutual information, I, is given by

$$I = \frac{M-1}{2}\log\left[1+\frac{S}{N}\frac{[1-\rho_S]}{[1-\rho_N]}\right]+\frac{1}{2}\log\left[1+\frac{S}{N}\frac{[1+(M-1)\rho_S]}{[1+(M-1)\rho_N]}\right].$$

As long as the signal is not totally correlated ($\rho_S < 1$), the information scales as the number of neurons, M. Correlations in the signal induce correlations in the response, so the information scales as M even when the neurons are correlated. For a completely correlated signal ($\rho_S = 1$) and uncorrelated noise ($\rho_N = 0$), the information scales as $\log(M)$. Finally, for a completely correlated signal ($\rho_S = 1$) but slightly uncorrelated noise ($\rho_N > 0$), the information asymptotes to a constant. In our poster we extend these results to non-Gaussian distributions, and discuss ways to compute information from neuronal populations in realistic experiments.

649.4

COMPUTING MEAN FIRING RATES OF ENSEMBLES OF REALISTIC NEURONS. A.G. Siapas, E. Todorov and D.C. Somers* MIT, E25-147A, Cambridge, MA 02139

Realistic models of neural circuits typically are too complex to be analyzed exactly and have too many parameters to be fully explored in computer simulations. Thus, methods for characterizing the collective dynamics of such systems are needed. Here, we develop a technique for approximating the mean firing rate of highly interconnected, homogeneous groups of neurons that receive Poisson input. This technique applies to single compartment neurons with realistic synaptic and intrinsic currents. Assuming only that conductances equal their averages, we derive a nonlinear equation relating the mean firing rate to the cellular and network parameters. This approach extends to the analysis of multiple neuronal groups, for which a system of equations is derived.

We compare our analytical results to simulations and observe a close fit for most parameter settings. In a system with 2 excitatory and 2 inhibitory groups with a total of 250 cells, the approximation error is less than 2%. The accuracy of mean firing rate calculations depends on proper estimation of the afterhyperpolarization reset potential. Unlike most related techniques which assume a constant reset potential, our method computes this potential as a function of input. The current form of our analysis is inaccurate only near threshold input levels where conductance variability significantly affects firing rate. We are presently extending the approximation technique to take this variability into account.

Computing the mean firing rate with our technique reduces to solving a system of nonlinear equations, which is much faster than full simulation. In addition to guiding the search for suitable model parameters, our technique also can yield analytical insights not available through simulations. For example, we can estimate the sensitivity of the mean firing rates to changes in model parameters. In addition, the system of equations can be solved directly to find parameter settings that achieve a desired set of mean firing rates.

649.5

SUBCORTICAL CONTROL OF SYNCHRONIZATION OF CORTICAL ACTIVITY: A MODEL. P. König, P.F.M.J. Verschure*. The Neurosciences Institute, San Diego, CA 92121.

A large body of experimental results suggest that the synchronization and desynchronization of neuronal activity is an important feature of cortical dynamics. These processes may serve binding and segregation of neuronal populations forming representations of perceived objects. An implication of this hypothesis is, that not only the level of neuronal activity, but also the level of coherence in the cortical network is regulated.

Here a model of the interaction of cortical areas with subcortical structures is presented, which addresses this issue. In the cortical network the distribution of synapses along the dendritic tree is captured by taking into account their finite electrotonic length. This parameter specifies the size of the set of effective synapses. The electrotonic length is influenced by modulatory transmitters released by the afferents of subcortical neurons, which are modeled to be very sensitive to synchronous input and are assumed to perform a spatial integration of cortical activity, thus forming a regulatory system for the level of cortical synchronization. These general principles have been applied to a model of the visual cortex demonstrating the adjustment of the interaction of cortical neurons to the degree of coherence present in the visual stimulus. Furthermore, this mechanism allows the regulation of the spatial scale of cortical processing. These results are in accordance with recent experimental observations (Munk et al. 1994, Neuroscience Abstracts), which demonstrate the involvement of the parabrachial nucleus in the regulation of cortical synchronization.

In conclusion, this study contributes to the evidence that dendritic processing forms an integral part of the function of neurons. Moreover subcortical structures can modify dendritic integration in cortical neurons and thus influence their degree of synchronization.

649.6

SINGLE UNIT RECORDING CAPABILITIES OF A 100 MICROELECTRODE ARRAY. C. T. Nordhausen*, E. M. Maynard, P. J. Rousche, R. A. Normann, Moran Laboratories, Department of Bioengineering, University of Utah, Salt Lake City, Utah 84112, USA

Recently, the focus of many cortical electrophysiological studies has switched from the conventional single microelectrode approach to multielectrode investigations. This has been the result of the interest in the parallel processing aspects of cortex, and the spatiotemporal firing patterns of ensembles of neurons. We have developed a three dimensional silicon electrode array, the Utah Intracortical Electrode Array, which provides 100 separate channels for neural recording in cortex.

In order to demonstrate the efficacy of the electrode array for intracortical recording, we have quantified various aspects of its acute and chronic recording capabilities. Our preliminary results from acute preparations indicate that 60 to 70% of the electrodes yield evoked neural activity. Histology indicates that the majority of the remaining electrodes do not yield neural activity because they are positioned over a sulcus. The amplitude of the responses recorded with the array range from 40 to 180 μV with background noise on the order of 10 to 15 μV. Although the signal to noise ratio of these recordings is not as high as in the case of single microelectrodes, we are able to identify, on average, 3.5 units for each electrode that has recordable activity using time and amplitude window discrimination techniques. We have identified and tracked the same units for up to 4 weeks postimplant in ongoing chronic implantations. These results indicate that the electrode array provides a stable substrate for long term neural recording in cortex.

The recording capabilities of the array combined with the large number of available electrodes allow for a wide range of potential applications. Long term studies of specific groups of neurons in cortex are possible because of the stability of the electrode array. The identification of single units make it possible to acutely record from hundreds of units simultaneously.

649.7

OPTIMAL POPULATION CODES FOR A FUNCTIONAL ANALYSIS OF VISUAL CORTEX DYNAMICS. M.A. Giese, A.C. Akhavan*, W. Erlhagen, D. Jancke, A. Steinhage, H.R. Dinse, G. Schöner. Institut für Neuroinformatik, Ruhr-Universität, Bochum, Germany and CNRS-LNC, Marseille, France.

Application of population codes for a simple reconstruction of the stimulus or effector response from neural response data does not reveal much insight in the functional mechanisms in the neural substrate. We redefine population code as a class of algorithmic schemes projecting a set of measured neural activities onto one or more interesting parametric dimensions. Specifying these dimensions by a task or stimulus makes collective neural response data usefully interpretable.

Regarding population coding as a mathematical estimation problem we construct an optimal population estimator for the neural activity distribution over an abstract parameter space. We use a linear population estimator with well defined statistical properties derived by generalization from the optimal linear estimator described by Salinas & Abott (*J. of Comp. Neurosc. 1: 89, 1994*). Estimated activity distributions from cat primary visual cortex can directly be compared to predictions derived from a neural field model on dynamical spatio-temporal interactions in the visual field. The model reproduces psychophysically observed attraction and repulsion effects. It yields predictions for the activation dynamics in the underlying neural ensemble, which are specific for the functional properties of the neural substrate.

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649.8

PRESERVED MOTION DISCRIMINATION IN MONKEYS WITH EARLY LESIONS OF STRIATE CORTEX. T. Moore, A.B. Repp, H.R. Rodman*, C.G. Gross. Department of Psychology, Princeton University, Princeton, NJ 08544.

The ability to discriminate visual stimuli on the basis of presence or direction of motion has been reported to survive damage to striate cortex in humans. In contrast, we previously reported (Moore et al., NS Abs. 19: 1801, 1994) that monkeys with unilateral striate lesions could not distinguish direction of motion in small (5 deg. diameter) dynamic random dot displays presented in the hemifield contralateral to the lesion. To address the possible role of stimulus size and retinal position in the residual visual capacity following striate cortex damage, we tested the ability of 3 monkeys with unilateral striate lesions made in infancy (5-6 weeks) to discriminate direction of motion in considerably larger (15 deg. diameter) displays of 0.5 deg. random dots centered at 13.5 deg. on the horizontal meridian. Magnetic resonance imaging indicates a complete lesion in one subject and only limited sparing of the peripheral field representation in striate of the other two. A go/no-go paradigm using a saccadic eye movement response was employed to test the monkeys' ability to discriminate upward from downward motion at 4 and 20 deg./sec. All monkeys learned the tested discriminations in both hemifields. The results suggest that in monkeys with striate cortex damage, some motion discrimination capacity is indeed preserved, at least after early lesions.

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649.9

STABILITY AND VARIABILITY OF VISUAL FIELD DEFICITS IN BRAIN DAMAGED PATIENTS, E. Kasten*, S. Wüst and B. A. Sabel. Inst. of Med. Psychology, Medical Faculty, Otto-von-Guericke Univ., Leipziger Str. 44, 39120 Magdeburg, Germany.

Thirtysix brain-damaged patients with visual field deficits were investigated to study the variability of the blind visual field (15 stroke, 9 trauma, 4 brain surgery, 3 inflammation, 4 cerebral hemorrhage, 1 cerebral degeneration). In addition to the assessment with the Tübinger automatic perimeter we used the computer programs PERIMAT (light detection), PERIFORM (form recognition) and PERICOLOR (color perception) for a more exact diagnostic of the the mid-section of the visual field up to 12.5° vertical and 20° horizontal eccentricity. Five repeated measurements were made in 5 independend sessions and the results were then compared. There was a surprising and reliable stability of visual field defects with correlations across the 5 sessions from r=0.86 up to r =0.94, depending on which diagnostic test was applied. Individual differences in physical health or mental condition, the age, the attention span, the cause of the lesion or the extent of the restricted visual field of the patients had no significant influence on the variability of visual field defects. Further analysis showed that most patients had no or very few islands of residual vision. 41.2% of stimulus locations in the blind area were only seen once, 27.1% twice, 18.8% three times, 8.2% four times and 4.7% in all 5 measurements. Perceived items to which the patient responded correctly only once or twice may perhaps be due to fixation instability or attentional deficits. In conclusion, there are only few reliable islands of residual vision in the blind area of most patients. [Supported by Kuratorium ZNS and DFG-No. Sa 433/6-1]

649.10

SPATIAL AND TEMPORAL FREQUENCY SENSITIVITY IN HUMAN STRIATE CORTEX MEASURED WITH FUNCTIONAL MRI (fMRI) J.B. Demb*, G.M. Boynton, S.A. Engel, J.D.E. Gabrieli, D.J. Heeger, and G.H. Glover Depts. of Psychology and Radiology, Stanford University, Stanford, CA 94305

Human spatial sensitivity depends on the temporal frequency of the stimulus. At high temporal frequencies (TF), sensitivity to sinusoidal gratings is best below 3 c/deg while at low TF, sensitivity is best between 3 and 6 c/deg. In addition, sensitivity to high spatial freqency (SF) gratings decreases with eccentricity. This study measured activation in human striate cortex (V1) to examine the neural correlates of pattern vision. We measured blood oxygen level-dependent (BOLD) fMRI signal in V1 while subjects viewed counterphase modulating full-field sine-wave gratings. We used a T2*-weighted gradient echo spiral sequence (1.5T, .78x.78x5mm voxel size, TR=75ms, TE=40ms, FA=23deg) and a cross-correlation analysis. Our results were consistent with psychophysical measurements. Low SF gratings produced best correlations with the stimulus at high TF while high SF gratings produced best correlations at low TF. Posterior regions of V1, close to foveal representations, were primarily activated by high SF/low TF stimuli. Anterior regions of V1, corresponding to peripheral representations, were primarily activated by low SF/high TF stimuli. These results may be a consequence of variations in receptive field size or a change in the relative amount of parvocellular and magnocellular input along V1.

Supported by Stanford OTL.

649.11

COMPARISON OF VISUAL CONTRAST-RESPONSE FUNCTIONS USING MAGNITUDE ESTIMATION PSYCHOPHYSICS AND FUNCTIONAL MAGNETIC RESONANCE IMAGING. M.R. Peters, B.J. Anderson*, and E.A. DeYoe, Dept. of Cell. Biol. & Anat., Med. Coll. of Wisc., Milwaukee, WI 53226.

Areas of the striate and extrastriate cortices which are responsive during a contrast scaling task were identified using functional magnetic resonance imaging (FMRI). For these areas, the relationship between perceived contrast and the magnitude of the FMRI signal was examined.

During gradient-recalled echo planar imaging, both the FMR signal and psychophysical data were simultaneously collected. Four human subjects were each placed in the scanner and allowed five minutes to adapt to the background luminance. During the trials they viewed sine-wave gratings varying in contrast from 5-100%. Subjects used simple hand signals to indicate estimated contrast from a modulus-free scale of their own choosing.

For each voxel, the psychophysical estimates of stimulus contrast and FMR response magnitudes were compared as a function of the physical stimulus contrast. For each of the 4 subjects, the estimates of stimulus contrast were linear with physical contrast when plotted in log-log coordinates, though for 1 subject a "ceiling" effect was observed at the highest contrasts. For some, but not all voxels in V1, the MR signal amplitude matched the psychophysical function. This variation may reflect differences in either the underlying neuronal response, the hemodynamic mechanisms responsible for the FMRI signal, or other as yet unidentified factors. However, the close correspondence of the psychophysical and FMRI responses for some voxels suggests that this approach can identify sites potentially responsible or supporting the perceptual events.

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649.12

VISUAL TEXTURE PERCEPTION IN HUMANS: fMR AND PET STUDIES. L. Beason-Held*, N. Azari, K. Purpura, L. Optican, J. Krasuski, M. Schapiro, S. Rapoport, J. VanMeter. Nat. Inst. of Health, Nat. Inst. on Aging, Lab. Neurosci., Bethesda, MD 20892.

To investigate cortical responses to a specific set of features within visual stimuli, isodipole textures were presented to 6 healthy young subjects during H215O positron emission tomography (PET) and blood oxygenation level dependent (BOLD) functional magnetic resonance (fMR) imaging. Two families of textures were used: Random textures in which the pattern of black and white checks were arranged in a random fashion, and Even textures in which the black and white checks were ordered to produce extended contours and rectangular blobs at multiple spatial scales. During fMR scanning, the subjects were presented with either Random or Even textures interwoven with alternating blocks of a control fixation condition. Relative to fixation, Random stimulation produced slight activation in the primary visual cortex (Area 17). In contrast, Even stimulation resulted in increased striate as well as extrastriate (Areas 18, 19) activation. As for the PET study, the subjects were given four scans during Random stimulation and four during Even stimulation. The results from this study support the fMR findings, in that Even stimulation increased blood flow in the primary and extrastriate cortices relative to Random stimulation. Together, these results suggest that early form processing is not limited to extrastriate cortex, and mechanisms in both striate and extrastriate cortex are sensitive to the ordered features present in the more visually salient Even textures.

649.13

CHANGES IN REGIONAL CEREBRAL BLOOD FLOW BY FLASH AND PATTERNED VISUAL STIMULATION IN MONKEY VISUAL CORTEX. K. Imamura*[1,2], H. Onoe[1,2], T. Shiomitsu[1,2], K. Onoe[1], H. Tsukada[1,3], T. Kakiuchi[3], and Y. Watanabe[1,2]. [1]Subfemtomole Biorecognition Project, JRDC, Osaka 565, [2]Osaka Bioscience Institute, Osaka 565, [3]Central Res. Lab. Hamamatsu Photonics K.K., Shizuoka 434, Japan.

We studied changes in the regional cerebral blood flow (rCBF) induced by visual stimulation using $H_2{}^{15}O$ and positron emission tomography (PET). Averaged subtraction images of different experimental conditions were made and signals were superimposed on magnetic resonance (MR) images after statistical evaluation(ref.). We found that (1) it was more difficult to obtain significant signals in the visual cortex under anesthetized and paralyzed condition than awake condition, (2) larger signal was obtained in binocular stimulation than monocular stimulation, (3) flash stimulation induced large signals in the visual cortex as moving line-pattern stimulation, (4) activity in the frontal region, including the cingulate cortex was decreased by visual stimulation, (5) visual stimulation applied to the deprived eye of monkeys that had been suffered monocular deprivation over the sensitive period still induced some responses in the visual cortex. These results show not only dynamic change in rCBF by visual stimulation but also dissociation between the generation of action potential and rCBF-change in the visual cortex.

(ref.) H. Takechi, et al. Neurosci. Lett. 182, 279-282 (1994)

649.14

IS THERE A 'FINAL COMMON PATHWAY' FOR THE TWO MONOCULAR VIEWS? T.J. Andrews*, L.E. White, D. Binder and D. Purves. Department of Neurobiology, Duke University, Durham NC 27710.

The "singleness" of human binocular vision is usually explained by the blending of information generated by each eye into a common stream that ultimately leads to perception. This scheme is exemplified by the phenomenon of stereopsis, which depends on binocularly driven cells in the primary visual cortex that are sensitive to spatial disparity. To ask whether the concept of monocular blending pertains to all aspects of vision, we repeated an experiment on flicker-fusion carried out by Charles Sherrington (Br. J. Psychol. 1: 26-60, 1904) nearly a century ago. The critical flicker-fusion frequency (CFF) is the frequency at which a flickering light is perceived as being fused 50% of the time. We determined the CFF for 25 subjects in two situations: (1) trains of light flashes presented synchronously to both eyes; and (2) the same trains presented asynchronously to the two eyes. If the independent views of the two eyes are fused in a binocular processing stream, then the critical flicker-fusion frequency should be much lower in the alternating presentation than in the synchronous presentation. One-second trains of flashes generated by two stroboscopic light sources were presented dichoptically; the frequency of flashes (20-65 Hz) and their temporal relationship were controlled by computer. Contrary to prediction, we found, as did Sherrington, only a small difference between the two conditions (mean synchronous CFF $\pm$ SD = 47.3 $\pm$ 4.1 Hz; mean asynchronous CFF $\pm$ SD = 46.3 $\pm$ 4.2 Hz). We conclude that binocular convergence at a cellular level, which is essential for some aspects of visual perception, may not be a general rule. Perhaps, visual perception in this circumstance derives from the activation of circuitry that elaborates monocular information independently.

649.15

EVALUATION OF PSEUDORANDOM M-SEQUENCES FOR HUMAN VISUAL EVOKED NEUROMAGNETIC RESPONSES. H.-W. Chen, C. Aine*, D. Ranken, E. Best, E. Flynn, and C.C. Wood. Biophysics Group, M715, Los Alamos National Laboratory, Los Alamos, NM 87545

The primary goal of this study is to assess the utility of using m-sequence stimuli, a general form of steady-state stimuli with broadband frequencies, in visual neuromagnetic studies. Three primary reasons for examining m-sequence stimuli are: 1) m-sequences are orthogonal to output noise (random or periodic) and may, therefore, enhance the signal-to-noise (S/N) ratio; 2) different m-sequences are orthogonal to one another and may, therefore, permit the simultaneous presentation of multiple stimuli at different locations, allowing for faster data acquisition; and 3) m-sequence results, in conjunction with nonlinear systems analysis, may provide information about the system's nonlinear dynamics. In this study, we evaluated the utility of m-sequences by comparing the S/N ratio per unit time obtained using both transient and m-sequence stimuli. In addition, the possibility of resolving two responses evoked by paired stimuli was also examined.

Evoked magnetic fields were recorded with a BTi 7-channel SQUID-coupled gradiometer system in a magnetically-shielded chamber. Experimental paradigms using both transient (266 ms duration and ~1 Hz rate of presentation) and m-sequence visual stimuli (with length 1023 at 30 Hz rate) were conducted. A single stimulus was presented at a single location (3^0), or pairs of stimuli were presented at three different eccentricities (0.8^0, 1.6^0, and 3^0) in the visual field. Results from three subjects show that the S/N ratio for the m-sequence study was 2-3 times higher than the transient study, for single stimulus placements, when total length of stimulus presentation time was equated across studies. The amplitude distributions across the 7 sensors were similar for transient and m-sequence stimuli. In addition, the time courses across the 7 sensors were not proportional to one another, suggesting the existence of multiple evoked visual sources. The response distributions evoked by the m-sequence pairs were quite similar to the distributions evoked by single stimuli. Thus far, it appears that m-sequence stimuli provide a useful approach for MEG/EEG studies of vision.

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649.16

HIGH-SPEED IMAGING OF NEURAL ACTIVITY IN THE TURTLE CORTEX EVOKED BY "MOVING STIMULI"

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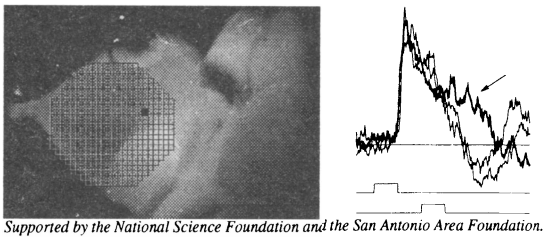
To examine the idea that the turtle dorsal cortex may be important in the detection of movement, visually-evoked activity in the cortex was monitored using combined multi-site optical recording and video imaging techniques. The image at left shows the outlined of a 464-element photodiode array superimposed on the unfolded cortical sheet using an isolated eye/brain preparation of the turtle, *Pseudemys scripta*. "Moving stimuli" were generated using a matched-pair of laser diodes (3 mW, 670 nm) that delivered two light spots (200 ms duration) under computer control. Optical signals at right show that depolarization of the dorsal cortex (filled box in grid) is longer (arrow) when visual stimulation was switched between a rostral and a caudal located spot (thick trace) than the responses observed when both light spots were delivered at the same visual location (thin traces).



Supported by the National Science Foundation and the San Antonio Area Foundation.

VISUAL CORTEX: STRIATE VIII

650.1

LAMINAR AND COMPARTMENTAL ORGANIZATION OF IMMUNOCYTOCHEMICALLY LOCALIZED AMPA RECEPTOR SUBUNITS IN MACAQUE PRIMARY VISUAL CORTEX. R.K. Carder*. Zanvyl Krieger Mind/Brain Institute and Dept. Psychology, Johns Hopkins University, Baltimore, MD 21218.

Fast excitatory synaptic neurotransmission in the visual system is mediated predominantly by α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptors. Cloning and expression studies have demonstrated that AMPA receptors are composed of multiple subunits with each subunit conferring unique pharmacological, kinetic, and gating properties. By use of subunit-specific antibodies, this study explored the organization of AMPA receptor subunits within the adult macaque primary visual cortex (V1). Immunoreactivity for each of the three AMPA subunits, GluR1, GluR2-3, and GluR4, consisted of rich plexuses of punctate profiles localized within the neuropil and to varying degrees somata and proximal processes. GluR1, GluR2-3, and GluR4 immunoreactivity was present through the thickness of V1, but was densest in three bands that corresponded to layers II - IVA, IVC and VI. Within different layers of V1 the immunostaining for AMPA subunits formed distinct patterns. In layer IiII, patches of intense immunostaining coincided with CO-rich blobs; in layer IVA, intense subunit staining formed a conspicuous honeycomb pattern; and in layer IVC subunit staining formed a very fine lattice. The principal observations of the present study are that the major subunit variants of the AMPA receptor are distributed in very similar patterns, with the greatest densities in zones of geniculocortical afferent terminations.

650.2

APPLICATION OF L-Arg AND L-NOArg MODIFY CELLULAR RESPONSES IN THE PRIMARY VISUAL CORTEX OF THE CAT. J. Cudeiro*, C. Rivadulla, R. Rodríguez, S. Martínez-Conde and C. Acuña. Departamento de Fisiología, Universidad de Santiago and Departamento de Ciencias de la salud I, Universidad de La Coruña, Spain.

Fibres arising from the brainstem provide cholinergic input to the lateral geniculate nucleus (dLGN), while the visual cortex receives a cholinergic input from the basal forebrain. Recent evidence has shown that nitric oxide synthase (NOS), synthetic enzyme for nitric oxide (NO), is co-localized with ACh in both pathways[1,2]. We have previously shown that NO is necessary for the transfer of visual information in the dLGN, gating visual input by an action involving NMDA receptors[3,4]. Here we extend these observations by examining the role of NO in the primary visual cortex.

Experiments were carried out on paralysed, anaesthetised adult cats (halothane 0.5-1% in N_2O and O_2 70:30; Gallamine 10 mgr/kg/h). Seven barrelled micropipetes were used for extracellular recording and iontophoretic ejection of drugs. The effect of application of L-NOArg (N^G-nitro-L-arginine, an inhibitor of NOS) and L-Arg (L-Arginine, the physiological substrate of NOS) was tested on spontaneous, visual and NMDA/AMPA evoked responses. In 14/40 cells application of L-NOArg produced a decrease both in spontaneous and visual responses, without changing visual specificity. This effect was reversed in 11/14 by co-application of L-Arg (itself without effect), and is therefore consistent with our data in the dLGN. Surprisingly, in 16/40 cells application of L-Arg abolished cell responses, even using very low ejection currents. This effect was unaltered by L-NOArg in any case. 25% of cells were unaffected by either drug, alone or in combination. There was no clear relationship between the effect seen and either cell type or laminar position. In a different block of experiments, we have observed that ejection of L-NOArg essentially suppresed both NMDA and AMPA responses in 40% of cells.

While it seems likely that a significant proportion of cortical cells respond to NO in a manner similar to the effect we have already demonstrated in the dLGN, the intriguing and potent action of L-Arg on a large number of cells remain unexplained.

1.-Bickford et al., (1993) J. Comp. Neurol. 334:410-430.
2.-Bickford et al., (1994) J. Comp. Neurol. 348:481-410.
3.- Cudeiro et al., (1994) J. Neurophysiol. 71:146-149.
4.- Cudeiro et al., (1994) Neuropharmacol. 33:1413-1418.

650.3

A Simple Model of Cortical Excitatory Cells Linking NMDA-Mediated Currents, ISI Variability, Spike Repolarization and Slow AHPs. T.W. Troyer* and K.D. Miller, W.M. Keck Center for Integrative Neuroscience, UCSF, San Francisco CA 94143

Integrate-and-fire models assume neurons integrate many fast synaptic inputs until threshold, then fire a spike that resets membrane voltage to rest. Such models can account for rate coding and classical receptive fields. However, their applicability to excitatory cells in cerebral cortex is challenged by the following observations: (1) interspike intervals (ISIs) have high variability, which is inconsistent with integration of many random synaptic events; (2) excitatory cortical cells typically do not repolarize near rest after spiking; (3) chronic infusion of APV blocks visual cortical responses, suggesting an important role for slow synaptic events; (4) specific blockade of fast excitatory transmission by chronic infusion of CNQX (specificity verified by iontophoresis) does not block visual responses in V1 (CNQX data from the laboratory of Michael Stryker).

Previous models have invoked a delicate balance of excitation and inhibition and perhaps (2) to explain (1). Motivated by (3) and (4), we incorporate NMDA receptors and negative feedback from a slow AHP along with (2) to arrive at a simple and robust single-cell model consistent with (1)-(4). Synaptic currents with a large NMDA-mediated component lift the membrane voltage near threshold. Incomplete repolarization returns the cell to this sensitive state. Because spikes result from small current excursions, the neuron has large gain and displays high ISI variability, as noted in previous models. Maintaining this sensitivity requires a balance of inward and outward currents, achieved here through slow, spike-evoked AHPs that compensate for changes in synaptic input. This adaptive balancing maintains wide dynamic range over long time scales despite high gain at short time scales. This model also qualitatively reproduces a peculiar bursting pattern displayed by a few well isolated units in the CNQX experiments. We are exploring possible functional roles these mechanisms could play in information processing on fast and slow time scales.

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650.4

SPATIAL ORGANISATION AND SELECTIVITY OF THE INTERACTING COMPONENTS UNDERLYING RESPONSES OF CELLS HIGHLIGHTING DISCONTINUITY OR INTERSECTIONS IN THE VISUAL WORLD.
H.E. Jones*, K.L. Grieve and A.M. Sillito. Dept. of Visual Science, Inst. of Ophthalmology, Bath St., London EC1V 9EL, UK.

Areas outside the receptive field excitatory discharge centre can exert very potent effects on visual cortical cell responses. These effects can be inhibitory, and are frequently linked to end-zones or side-bands, or both, although in some cases they may effectively surround the field (DeAngelis et al. 1994, *J. Neurophysiol.* 71:347-374; Li & Li 1994, *Vision Res.* 34:2337-2355). Facilitatory and/or disinhibitory effects can also be elicited from outside the excitatory discharge centre but there is little evidence regarding their spatial organisation. These influences are brought into strong focus by particular configurations of stimuli overlying the classical receptive field and the surrounding area of visual space. In some cells they may reflect a network effect serving to signal orientation discontinuity or intersections. Here we report evidence from primate V1 showing that both the facilitatory/disinhibitory and inhibitory influences are strongly dependent on the direction of stimulus motion, as well as the relative orientation, of stimuli driving the classical receptive field and surrounding areas of visual space. Areas producmg facilitatory effects are not necessarily uniformly distributed around the receptive field and may be very localised. For example the response to an optimally oriented grating patch overlying the classical receptive field may be most strongly enhanced by an additional orthogonally oriented patch, drifting in one direction only and displaced to a location adjacent to one corner of the field. The spatial localisation, directionality and orientation requirements for the secondary stimulus suggest a mechanism in V1 focusing on very specific facets of the visual world, for example the vectors of motion associated with an intersection such as a corner moving through space. They emphasise the need to dissect the functional organisation of primate V1 with more complex stimulus configurations.

650.5

EFFECTS OF METABOTROPIC RECEPTOR AGONISTS IN THE FELINE VISUAL CORTEX. P.M. Venters and A.M. Sillito. Dept. of Visual Science, Inst. of Ophthalmology, Bath St., London EC1V 9EL, UK.

We were interested in effects following from the Group 1 phosphoinositide linked metabotropic receptors (mGluRs) in the visual cortex, and particularly actions that derived from modulation of the potassium channels underlying the M current, because this might provide a means of local modulation of excitability superimposed on cholinergic influences in the neocortical circuitry. To this end we used microiontophoretic techniques to explore the action of a range of mGluR agonists on the visual and drug driven responses of cells in layers II-VI of the cat primary visual cortex. To our surprise we saw only a few examples of the type of facilitatory effects previously associated with the cholinergic modulation of the M-current (Sillito and Kemp 1983, *J. Physiol.* 250:287-304; Murphy *et al.* 1993, *J. Neurophysiol.* 69:1465-1474) and these were on cells in layers V and VI.

The predominant effect (90% of the cells) was a selective suppression of visual driving. Thus for example iontophoretic application of the multiple mGluR receptor agonist 1S,3R-ACPD depressed or blocked the visual response of cortical cells to an optimally oriented moving bar, but did not influence the responsiveness of the cells to iontophoretic pulses of NMDA or AMPA. Thus the post synaptic excitability of the cells to the agonists acting at the postsynaptic glutamate receptors was not changed whilst the visually driven response was. This effect seems most logically explained by a presynaptic effect mediated via Group 2 mGluRs. We observed it in cells throughout layers II-VI and its prevalence suggests that presynaptic control of the transfer of visual information may play an important functional role in the microcircuitry of the visual cortex.

650.6

MOLECULAR COMPONENTS OF THE cGMP SECOND MESSENGER SYSTEM ARE PRESENT IN RAT VISUAL CORTEX

D.Samanta Roy*, P.A. Kingston, and C.J. Barnstable, Interdepartmental Neuroscience Program, Dept. of Ophthalmology and Visual Sciences, Yale University School of Medicine, New Haven, CT 06510

Retrograde messengers such as nitric oxide (NO), and some of the transmitters implicated in learning and memory, as well as in synaptic changes during development, have been shown to affect the concentration of cGMP. cGMP exerts its effects through three known pathways, namely 1) cGMP-gated cation channels, 2) cGMP dependent protein kinases, and 3) cGMP regulated phosphodiesterases. We have presented a model to suggest how these might interact to modulate cell excitability (Ahmad *et al*, Neuron 12: 155, 1994). We wish, however, to determine the general applicability of this model to the brain, in particular the visual cortex.

To begin to study how cGMP might affect development and plasticity in mammalian visual cortex, we have examined the distribution and developmental expression of a series of molecules involved in cGMP metabolism and cGMP effector molecules. Using RT-PCR, we have detected expression of the cGMP-gated cation channel, cGMP kinase, cGMP stimulated phosphodiesterase, and the 61 and 63 kD forms of the calcium-calmodulin dependent phosphodiesterase in adult rat visual cortex. Preliminary in situ data using digoxigenin-labelled RNA probes for the rod cGMP-gated cation channel, suggests a rostral to caudal gradient of expression in the rat cortex, with the highest level appearing in the occipital cortex. Moreover, there appears to be only a subset of neurons labelled with the densest staining in layers II/III and V/VI.

In summary, we have found many of the molecular components of the cGMP second messenger system to be present in adult rat visual cortex, and have found the cGMP-gated channel to be localized to discrete layers of the visual cortex. These findings, together with the known effects of NO and cGMP in other brain regions, suggest an important role for the NO/cGMP system in visual cortical function.

Supported by grants from the NIH.

650.7

DISTRIBUTION OF ADENOSINE A1 RECEPTORS IN PRIMARY VISUAL CORTEX OF MONKEY

S. Larocque*, R.Z. Cohen, V. Zhao, and A. Chaudhuri
Dept. of Psychology, McGill University, Montreal, Canada

The adenosine A1 receptor has been implicated in a variety of physiological functions, including inhibition of neurotransmitter release. Adenosine binds to A1 receptors with high affinity and inhibits adenyl cyclase. The distribution of this receptor in visual cortex has not been previously reported.

Receptor binding in the primary visual cortex of normal and monocularly-deprived vervet monkeys was determined by in vitro autoradiography with the agonist [^{3}H]-CCPA. Thaw-mounted sections (15 μm) were treated to the radiolabeled ligand for three hours at room temperature. Non-specific binding was estimated on adjacent sections by adding 50nM cyclopentyladenosine in the incubation medium. Standard washing protocols were then followed and the sections were air dried. The slides were exposed to Hyperfilm-^{3}H (Amersham) at room temperature for 10 days, along with plastic standards of ^{3}H (Amersham).

We have found intense labeling in layer V and moderate labeling in the supragranular layers of primary visual cortex (area 17 or V1). The layer V distribution was especially striking because of contrast with the poor labeling in layers IV and VI. This pattern was unchanged in animals that had a monocular occlusion for up to six days. There was no evidence of modulation in receptor density in either set of ocular dominance columns.

Supported by grants from MRC (MA-12685) and NSERC (OGP0155482).

650.8

MODULATION OF SYNAPTIC INPUTS TO VISUAL CORTICAL NEURONS BY ADENOSINE AND 5-HT

Juan Varela, Kathryn Richards and Sacha B. Nelson* Dept. of Biology, Center for Complex Systems, Brandeis University, Waltham MA 02254

Although receptors for a variety of neuromodulators including serotonin (5-HT) and adenosine (ADE) are present in the visual cortex, their physiological actions have mostly been inferred from studies in other preparations. In order to study these effects directly, we made whole-cell voltage and current clamp recordings from pyramidal neurons in layers 2/3 and 5 of rat primary visual cortical slices, under visual control, using infrared videomicroscopy. ADE (10-100 μM) reduced the frequency of spontaneous EPSCs from 1.18 to 0.33 Hz without apparent change in their amplitude. EPSCs evoked by local intracortical stimulation were decreased by 40-75%. For stimuli which evoked minimal (apparently unitary) responses, ADE dramatically increased the failure rate. ADE also enhanced the synaptic facilitation and decreased the synaptic depression evoked by brief stimulus trains (5 @ 20 Hz).

Effects of 5-HT (10-50μM) and the specific 5-HT1B agonist CGS 12066A (20μM) were more heterogeneous. Spontaneous EPSC frequency and amplitude were not significantly affected (n=6). Minimally evoked EPSCs were reduced in 3/6 cells tested, and failures were increased (2/6). 5-HT at these doses did not directly alter neuronal excitability as measured by holding currents and the threshold for antidromic activation. These results suggest that ADE and 5-HT may act presynaptically in the visual cortex but may differ in their specific synaptic targets.

650.9

VISUAL DEPRIVATION DOES NOT ALTER MUSCARINIC RECEPTOR PROTEIN DISTRIBUTION IN AREA 17 OF RHESUS MONKEYS. M. Tigges, J. Tigges*, H.D. Rees, D.B. Rye and A.I. Levey. Yerkes Primate Center and Dept. of Neurol., Emory Univ., Atlanta, GA 30322.

Acetylcholine has been implicated in the normal function of visual cortex and in ocular dominance plasticity. We used antibodies to muscarinic cholinergic receptor proteins m_1 and m_2 to investigate the expression of these proteins in area 17 of a normal rhesus monkey and of 2 monkeys raised with a black contact lens on one eye from birth and 2 monkeys with long-term monocular eyelid suture as adults. In area 17 of the control brain, immunoreactivity of both proteins was distributed in a complex laminar pattern. Adjacent cytochrome oxidase sections were used to confirm assignment of immunoreactivity to various layers. m_1 was densest in layers 6 and 2/3, followed by layer 5. Layer 4 contained several bands of different intensities. A thin light band of reaction product ran through 4A, 4B reacted darker; another thin, but very light band occurred at the 4C/5 border. The remainder of 4C was moderately light. In contrast, m_2 reactivity was densest in lower layer 4C. The honeycomb pattern of 4A was reactive and layers 2/3 exhibited alternating dense and light regions. Layer 5 was slightly lighter than 4C, and layers 4B and 6 were stained the least. m_1 antibody reacted predominantly with neurons, m_2 antibody labeled mostly processes. In area 17 of visually deprived monkeys, the distribution of the 2 receptor proteins appeared normal, except that in one lidsutured monkey a thin band at the 4C/5 border reacted more densely with m_2 antibody than 4C. Thus, these proteins seem to be insensitive to monocular elimination of visual input via occlusion from birth and to long-term elimination of pattern vision imposed on the adult visual system.

Supported by NIH grants EY09737, RR00165, and NS30454.

650.10

NEUROCHEMICAL ORGANIZATION OF MACAQUE STRIATE CORTEX: CORRELATION OF CYTOCHROME OXIDASE WITH NOS, NADPH-DIAPHORASE, NMDAR1, AND Na^+K^+ATPASE. M. Wong-Riley*, W. Liebl and Z. Huang, Dept. of Cellular Biology & Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

Previous studies indicate that a) cytochrome oxidase (C.O.)-rich puffs in the macaque striate cortex sustain a higher level of maintained neuronal activity than interpuffs, b) neuronal and synaptic properties differ between these two compartments, and c) asymmetric, glutamate (Glu)-immunoreactive synapses predominate in puffs. A major Glu receptor family is NMDA, which is implicated in the stimulation of nitric oxide synthase (NOS) and in the production of NO, a gaseous intra- and inter-cellular messenger (Garthwaite, 1991; Dawson & Snyder, 1994). To determine if these neurochemicals are enriched in puffs, we processed serial frozen sections of the monkey visual cortex for C.O., NOS, NADPH-diaphorase, NMDAR1, and Na^+K^+ATPase (a major energy-consuming enzyme), respectively. We found that they all have a heterogeneous pattern of distribution in the supragranular layers that centered on puffs, but the labeling of NOS was the lightest. In addition, the staining of all five were intense in layer 4C and, with the exception of NOS, exhibited a honeycomb pattern in 4A. At the EM level, the density of NOS-positive immunogold particles was significantly higher in C.O.-rich type C cells than in the other cell types of puffs. Thus, our results are consistent with an excitatory synaptic circuitry in puffs that involves glutamate and NMDA receptors, as well as nitric oxide in selective cell types. The excitatory synaptic activities are likely to impose a higher energy demand in puffs than in the surrounding interpuffs.

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650.11

EFFECTS OF MONOCULAR IMPULSE BLOCKADE ON GABA IMMUNOREACTIVE NEURONS IN CYTOCHROME OXIDASE (C.O.)-RICH PUFFS OF THE MACAQUE STRIATE CORTEX: QUANTITATIVE EM ANALYSIS. F. Nie, E. Godfrey* and M. Wong-Riley, Dept. of Cellular Biology & Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226

Previously, we have shown that GABA-immunoreactive (GABA-IR) neurons in supragranular puffs of the monkey striate cortex have high levels of cytochrome oxidase (C.O.) activity. We now have examined the effects of 2 weeks of monocular TTX treatment on these neurons in activity-deprived and non-deprived puffs (DP and NP). In ultrathin sections of DPs doubly labeled for C.O. and GABA, GABA-IR cells and GABA-IR terminals showed a significant shrinkage in their mean size ($P<0.05$ for both), a drastic reduction in the proportion of dark and moderately C.O.-reactive mitochondria, and a significant decrease in the number of GABA-immunogold particles as compared to comparable cells in NPs. The densities of GABA-IR axon terminals and GABA-IR symmetric synapses in DPs were also significantly reduced ($P<0.01$), while those of GABA-IR cells and dendrites were unchanged. The total number of synapses in DPs and NPs was constant. Non-GABA-IR cells exhibited relatively minor changes in these parameters. These results suggest that: (1) metabolic activity and GABA levels in GABA-IR neurons are regulated by neuronal activity in adult monkey striate cortex; and (2) GABA-IR neurons are much more vulnerable to functional deprivation than non-GABA-IR neurons, presumably because their cell bodies are more dependent upon sustained excitatory input. (Supported by NIH EY05439 & NS18122).

650.12

MUSCIMOL AND BACLOFEN DIFFERENTLY SUPPRESS RETINOTOPIC AND NON-RETINOTOPIC CORTICAL RESPONSES. K. Mizobe, E. Schmidt and T. Kasamatsu* The Smith-Kettlewell Eye Res. Inst., San Francisco, CA 94115

The locally recorded field potentials elicited by contrast reversal of bar gratings have extremely large receptive fields beyond the usual range of the classical receptive field of single units at the same site. They have two distinct components: the retinotopic fast-local component (FLC) and the non-retinotopic slow-distributed component (SDC). The latter is thought to be a physiological correlate of the long-range lateral connections (Kitano et al., 1994). Earlier, we have shown that muscimol, a $GABA_A$ receptor agonist, suppressed the FLC more strongly than the SDC though its effects on the latter were detected earlier than those on the former (Mizobe, et.al., 1995).

In the present study, we compared the suppressive effects of muscimol with those of a $GABA_B$ receptor agonist, baclofen. Visual stimuli were contrast reversal of achromatic bar gratings in a 5-deg patch placed at either retinotopic locus or ~15 deg in the periphery. Field potentials were recorded by a pair of microelectrodes implanted in area 17 near the projection of the area centralis. Responses were extracted by a multi-input nonlinear systems analysis method (Sutter, 1992). Muscimol (10mM) or baclofen (5mM) was continuously infused for 2.3~5.6 hours at a rate of 5 µl/h through a 30-G cannula implanted ~2 mm posterior to the recording site.

Within 1 hour after the start of infusion, both muscimol and baclofen decreased the amplitude of the SDC, but not the FLC. Thereafter, however, the FLC amplitude was more strongly decreased than the SDC amplitude throughout the muscimol infusion. By contrast, the SDC was more strongly suppressed by baclofen than by muscimol. The suppressive effect of baclofen tended to be stronger on the SDC than the FLC, but the difference did not reach a significant level. The present findings suggest that : 1) the retinotopic responses are controlled more strongly by $GABA_A$ receptors than $GABA_B$ receptors, and 2) the reverse is true for the non-retinotopic responses.

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650.13

SEROTONIN FACILITATES SYNAPTIC PLASTICITY IN KITTEN VISUAL CORTEX — AN IN VITRO STUDY. Q. Gu*, L. Kojic, R.M. Douglas and M.S. Cynader, Department of Ophthalmology, University of British Columbia, Vancouver, BC, Canada V5Z 3N9

We have investigated the hypothesis that serotonin is involved in activity-dependent synaptic modification in the developing visual cortex. Visual cortex slices (400 - 500 µm) were made from kittens aged between 40 - 57 days. At these ages several subtypes of serotonin receptors are found to be expressed transiently at higher level within the visual cortex. Field potentials in layer IV evoked by white-matter stimulation were recorded. Normally, low-frequency white-matter stimulation at 1 Hz for 15 minutes had no effects on field potentials in layer IV (10 out of 10 slices). However, when serotonin was present in the bath (1 µM) during the low-frequency stimulation, about half of the slices tested developed long-term depression (LTD) (5 out of 11 slices). When serotonin concentration in the bath was increased to 10 µM, the same low-frequency stimulation could induce either long-term potentiation (LTP) (3 out of 9 slices) or LTD (3 out of 9 slices). These results suggest that serotonin facilitates input-dependent synaptic modifications in kitten visual cortex. The differential effects mediated by serotonin (LTD, LTP, or no change) may depend on serotonin receptor subtypes located at the recording sites, since it has been shown that at these ages serotonin 5-HT2c receptors, for example, are localized in a columnar fashion across layer IV.

650.14

GABA iontophoresis modifies the intrinsic signal orientation map in cat area 18. L.J. Toth*, D.-S. Kim, S.C. Rao, M. Sur.
Dept. Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

While intracortical inhibition clearly has a role in generating orientation selectivity in visual cortex, how the balance of excitation and inhibition regulates orientation specificity remains unknown. To examine the issue, we injected GABA into a column of known orientation preference and imaged intrinsic signals to ascertain changes in orientation strength and preference in the surrounding cortical region. At the maximal concentration used (1.0M, 93nA) we were able to affect sites up to 1.5 mm distant from the injection. Sites with similar orientations to that at the injection showed a shift in orientation preference *away* from the injection site orientation. Orientation preference at the injection site changed up to 45°, although general activity levels were much lower in this region. These effects were reversible. Surrounding columns of orthogonal orientation, as well as more distant columns of all orientations showed no obvious changes during GABA application. Changing the amount of GABA ejected produced changes in the magnitude of the orientation shift, and the extent of cortex involved. While these results do not favor a strong role for cross-orientation inhibition, they do suggest that selective activation of inhibitory circuitry can profoundly alter the layout of orientation domains in the cortex, perhaps by selectively altering the balance of converging excitatory inputs to a region. Supported by EY07023.

650.15

THIP PRODUCES PROFOUND, SELECTIVE CHANGES IN FLASH EVOKED POTENTIALS OF HOODED RATS. B.E. Hetzler* and H.L. Zeisset, Dept. of Psychology, Lawrence Univ., Appleton, WI 54912.

Flash evoked potentials (FEPs) are useful in assessing the functional integrity of the visual system, although interpretation of any changes is difficult since little is known about the neuronal processes responsible for peak generation. Gamma amino butyric acid (GABA) is an inhibitory neurotransmitter believed to play a major role in information processing in the cerebral cortex, but its role in the elaboration of the rat FEP is unclear.

FEPs were recorded from the visual cortex of hooded rats at 5, 20, and 35 min. following i.p. injections of saline, and of 8, 16 and 24 mg/kg THIP on separate days. THIP is an analogue of muscimol, a GABA-A agonist. Most significant effects occurred at the 20 and 35 min recording intervals for both the 16 and 24 mg/kg doses, with effects at the 24 mg/kg dose the most pronounced. P1 amplitude remained unchanged, while N1 was reduced to such an extent that it became positive, ultimately blending into the rising phase of a novel positive component. This novel component had a latency of about 6 msec longer than N1, and became larger than P1 at the 24 mg/kg dose. P2 amplitude was drastically reduced, becoming negative. In contrast, components N2 and P3 were augmented, while the amplitude of N3 was unchanged. Latencies of most components were increased, with the late components increased to the greatest extent. A mild hypothermia of about 0.5 °C and 0.75 °C was observed at 16 and 24 mg/kg, respectively. The amplitude data suggest that the GABA-A receptor system is involved in the generation of the middle FEP components. However, the effects of THIP on the rat FEP also suggest that the role of GABAergic inhibition in FEP production is qualitatively different in the rat than in other species (e.g., cats; Zemon et al., in Evoked Potentials, Alan R. Liss, 1986).

651.1

CHARACTERIZATION OF CHLORIDE CURRENTS RECORDED FROM DISSOCIATED RAT TASTE CELLS. Xiao-Dong Sun* and M. Scott Herness, Indiana University School of Medicine, Muncie, IND. 47306.

We have characterized previously unidentified chloride currents from rat taste receptor cells using circumvallate and foliate papillae dissociated with papain using the patch clamp technique in the whole cell configuration. Chloride currents were identified as outward currents under conditions of 159 mM [Cl⁻]ext and 34 mM [Cl⁻]int by pharmacological isolation with 500 μM DIDS or 100 μM Niflumic acid. Currents reversed close to the expected Nernst potential and were on the order of a few hundred picoamperes in magnitude, increasing with more positive step potentials. When external chloride was lowered by isethionate or gluconate substitution, currents were reduced and the reversal potential shifted accordingly. Further isolation of these currents by blocking potassium currents with a combination of TEA and 4AP provided similar results. Chloride currents were additionally recorded as inward tail currents that appeared as inwardly rectifying at membrane potentials negative to the predicted chloride reversal potential. They were sensitive to both 500 μM DIDS and to 100 μM Niflumic acid as well as lowering external chloride concentration by ion substitution. Moreover they exhibited an inactivation that had temporal and voltage dependent components to it.

Supported by NIH DC00401.

651.2

RESPONSES OF MUDPUPPY TASTE RECEPTOR CELLS TO DENATONIUM. T. Ogura, A. Mackay-Sim[1] and S. C. Kinnamon*, Dept. Anatomy and Neurobiology, Colorado State Univ., Fort. Collins, CO 80523, the Rocky Mountain Taste and Smell Center, Univ. of Colorado Health Science Center, Denver, CO 80262 and [1] Faculty of Science and Technology, Griffith Univ., Brisbane, QLD 4111, Australia.

Denatonium (DN) is a potent bitter substance. Responses of isolated mudpuppy taste receptor cells to DN benzoate were studied by Ca^{2+} imaging with fura-2 and whole-cell recording. We identified receptor cells by staining the apical surface with FITC-WGA prior to isolation. In 87% of receptor cells, DN (5 mM) increased $[Ca^{2+}]i$ by 56.8 ± 8.8% from a resting level of 75.5 ± 2.8 nM (n = 26). The response always began in the apical tip of the receptor cell, even though the DN was applied to the entire cell. The response to DN was present in a Ca^{2+}-free external solution, but was reduced in magnitude by approximately 75%. Thapsigargin (1 μM), a Ca^{2+}/ATPase inhibitor, induced responses of similar magnitude to DN and inhibited subsequent responses to DN. Ryanodine (10 μM) had no direct effect on $[Ca^{2+}]i$ and failed to block the DN responses. These results suggest that DN increased $[Ca^{2+}]i$ mainly from thapsigargin-sensitive, ryanodine-insensitive Ca^{2+} stores in the majority of taste cells. A small subset of DN-sensitive cells also responded to caffeine (20 mM), suggesting that caffeine-sensitive Ca^{2+} stores may also contribute to bitter transduction in some taste cells Whole-cell recording showed that DN potentiated outward currents in most taste receptor cells. In some receptor cells and in all non-receptor cells, DN either blocked, or had no effect on, outward currents. Pharmacological experiments indicated that the DN-increased outward currents were carried by K^+ and Cl^- ions. Taken together, these data suggest that DN increases $[Ca^{2+}]i$, which subsequently activates Ca^{2+}-dependent K^+ and Cl^- currents in taste receptor cells.

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651.3

VERTEBRATE TASTE RECEPTOR CELLS HAVE NOVEL CATIONIC CHANNELS ACTIVATED BY QUININE. Takashi Tsunenari[1], Yukako Hayashi*[1], Manabu Orita[1], Takashi Kurahashi[2], Akimichi Kaneko[3] and Tomohiko Mori[1]. [1] Res. Ins. for Food Science, Kyoto Univ., Uji, Kyoto 611, Japan. [2] The National Ins. for physiological Sciences, Myodaiji, Okazaki 444, Japan. [3] Dep. of Physiology, Keio Univ. Sch. of Med., Shinjuku, Tokyo 160, Japan.

Quinine is known to be a bitter substance for various vertebrates. Whole-cell recordings under voltage-clamp were made from isolated taste receptor cells of bullfrog (*Rana catesbeiana*) while quinine hydrochloride was applied through a puffer pipette. Quinine stimulation elicited an inward current at the resting potential. When the blocking effect of quinine on K^+ currents was eliminated by Cs^+ pipette, the current-voltage relation was linear (the reversal potential; +16mV) and showed a clear increase in membrane conductance by quinine stimulation. The quinine-activated conductance was cation selective. The relative permeability ratio was $P_{Na} : P_{Cs} : P_K = 1 : 0.42 : 0.5$. The quinine-activated cationic conducting channel was suppressed by Ca^{2+}, sharing a common feature with quinine responses *in situ*. We therefore conclude that this novel cationic channel plays a crucial role in the bitter perception. Bitter-induced depolarization in taste cells has been explained by a blocking effect on K^+ channels. It, however, seems likely that taste receptor cells have, at least, two independent pathways for the detection of a bitter substance.

651.4

SODIUM SELF-INHIBITION IN AMILORIDE-SENSITIVE SODIUM CHANNELS: IMPLICATIONS FOR SALT TASTE TRANSDUCTION Timothy A. Gilbertson* and Huai Zhang. Pennington Biomedical Research Center, Louisiana State University, Baton Rouge LA 70808.

The role of amiloride-sensitive sodium channels (ASSCs) in salt taste transduction has been well-established. Recent experiments in fungiform taste buds have revealed that sodium transport into taste receptor cells (TRCs) is saturable, highly selective, regulated by hormones and inhibitable by amiloride and a number of its analogs. Thus, ASSCs in fungiform TRCs apparently respond to the same regulatory cues which govern their function in other transporting epithelia. In the present study, we are investigating the regulation of ASSCs in rat TRCs by extracellular sodium ions using patch-clamp and transepithelial current recording techniques. In isolated fungiform TRCs which contain ASSCs, we have recorded the current through ASSCs in response to voltage ramps using the perforated patch recording configuration while varying extracellular sodium concentrations. In most cells, currents through ASSCs are larger in 35 mM NaCl than in 70 mM NaCl, consistent with an inhibitory effect of extracellular sodium. This process, termed sodium self-inhibition, takes on the order of seconds to develop. Isolated TRCs which lack ASSCs do not demonstrate the sodium self-inhibition phenomenon. In both isolated TRCs and intact lingual epithelia, the sulfhydryl reagent, *p*-hydroxymercuribenzoate (*p*-HMB; 200 μM), removes inhibition by extracellular sodium, effectively increasing sodium currents. The effect of *p*-HMB is partially reversible by treatment with cysteine (10 mM). These results, taken together with the fact that in rats salivary sodium concentrations are ~60 mM, would imply that sodium self-inhibition may be an important regulatory process in fungiform TRCs and, hence, in the transduction of salty taste stimuli.

651.5

ELECTROPHYSIOLOGICAL ACTIONS OF QUININE ON DISSOCIATED RAT TASTE RECEPTOR CELLS. Yushe Chen* and M. Scott Herness, Indiana University School of Medicine, Muncie, IND. 47306.

The electrophysiological actions of quinine, a classic bitter stimulus, were studied on dissociated rat taste receptor cells from foliate and circumvallate papillae using patch-clamp techniques in the whole cell recording configuration. Its most profound action was a dramatic inhibition of outward potassium currents. Inhibitions could be measured from 10^{-6} to 10^{-3} M, a concentration range that coincides well with behavioral observations in rats. At the higher range inhibition of potassium currents was both complete and reversible (97% inhibition at 10^{-4} M). At lower concentrations inhibition was more profound on the sustained portions of the current, leaving a transient voltage-dependent current. This may suggest a site of action on delayed rectifiers. Input membrane resistance, on the order of 10 GΩ was increased by quinine by 10 - 20%. We tested for the presence of a quinine-induced current but none could be measured. Actions on sodium and calcium currents could also be measured. Quinine inhibited sodium current by 20 - 30% but increased calcium currents (measured with barium as a current carrier). Under current clamp conditions, some broadening of the action potential was noted in the presence of quinine. The action potential amplitude was decreased but the threshold was not changed.

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651.6

RESPONSES TO GLUTAMATE IN ISOLATED RAT TASTE CELLS. A. Bigiani, R. J. Delay, N. Chaudhari, S. C. Kinnamon and S. D. Roper.* Rocky Mt. Taste & Smell Center, Denver, CO 80262, and Dipt. Scienze Biomediche, Università di Modena, 41100 Modena, Italy.

Glutamate is an important taste stimulus for humans and animals alike. A metabotropic glutamate receptor, mGluR4, is selectively expressed in rat taste buds and may be responsible, in part, for transducing glutamate taste (Chaudhari, *et al.* '94). Yet, the mechanism of chemosensory transduction for glutamate is still unknown. We have recorded responses from single cells in isolated rat taste buds, using perforated patches. 20 mM L-glutamate was dissolved in a modified Tyrode solution (maintaining iso-osmotic solutions), and bath-applied to taste cells. Applying glutamate reduced the inward holding current at -80 mV by 5.3 ± 2.9 pA, (mean ± SD; n=3). The glutamate response decreased upon depolarization, with an apparent reversal potential of -11 ± 3 mV (n=2), close to the equilibrium potential for Cl^- (-15 mV). An increase in cell input resistance was observed during the glutamate response (4.7 → 10.1 GΩ, n=1). Glutamate had no effects on voltage-gated inward Na^+ and outward K^+ currents. Applying 0.85 mM L-AP4, a selective agonist for mGluR4, mimicked the effects of glutamate. However, at this concentration, the responses were much smaller. These data suggest that the activation of mGluR4 and the consequent modulation of a Cl conductance may be key events in taste transduction for glutamate.

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651.7

CHARACTERIZATION OF BITTER RECEPTORS THAT ACTIVATE α-GUSTDUCIN AND TRANSDUCIN IN TASTE MEMBRANES. L. Ruiz-Avila* and R.F. Margolskee,Roche Institute of Molecular Biology, 340 Kingsland Street, Nutley, NJ 07110.

Bitter and sweet tastes are believed to be transduced through heterotrimeric G proteins, which couple taste cell membrane receptors to intracellular effector enzymes. The taste receptors have not yet been functionally characterized nor molecularly cloned. Taste cells preferentially express a few G protein alpha subunits; notably transducin and gustducin (which is absolutely taste specific). These two proteins are closely related at the amino acid level, and have been shown to activate a taste-specific phosphodiesterase (Ruiz-Avila et al. 1995), thereby regulating the concentration of cyclic nucleotides inside the taste cells and consequently modifying the activity of taste cell cyclic nucleotide-regulated channels (Kolesnikov and Margolskee, 1995). We have reconstituted a taste responsive system using bovine or murine circumvallate papillae membrane preparations and purified bovine rod transducin or recombinant gustducin as exogenously added reporters. Both transducin and gustducin are specifically activated by receptors present in taste membranes and this activation is enhanced by the bitter compound denatonium (100 μM to 10 mM). Transducin/gustducin activation by denatonium-responsive receptors can be competitively inhibited with a peptide derived from the receptor interaction domain of transducin. The denatonium receptor-G protein interaction also requires βγ subunits. Mutations in the C-terminus of gustducin affect its interaction with rhodopsin and modify its interaction with the denatonium responsive taste receptor. L. R-A is a Fulbright scholar from the Spanish MEC-Fulbright postdoctoral program.

651.8

MULTIVARIATE CLASSIFICATION OF RAT TASTE RECEPTOR CELLS BASED ON ULTRASTRUCTURAL MEASURES OF NUCLEAR MORPHOLOGY. D. W. Pumplin, C. Yu, A. Z. Murphy*, and D. V. Smith, Dept. Anatomy, Univ. Maryland Sch. Medicine, Baltimore, MD 21201-1559.

Mammalian taste receptor cells are organized within taste buds on the tongue and other oral, pharyngeal, and laryngeal epithelia. These cells have been classified into light, dark, or intermediate cells on the basis of several ultrastructural criteria. Light cells are said to have large, round or ovoid, smooth nuclei with little heterochromatin and a relatively electron-lucent cytoplasm. Dark cells are characterized as having elongated, invaginated, heterochromatic nuclei and electron-dense cytoplasm. Intermediate cells lie between these extremes. Both the occurrence of large numbers of intermediate cells and the dependence of the light/dark categorization on the parameters of fixation suggest that such a classification may be somewhat arbitrary. Rats were perfused with 1% paraformaldehyde/2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer; tongues were removed, and semi-serial thin sections were cut transverse to the longitudinal axis of the vallate taste buds. To develop objective criteria for cell classification, the nuclear morphology of 95 cells was quantified with several measures that are purported to correlate with the light/dark cell classification (nuclear area, eccentricity, irregularity, and heterochromaticity). These measures discriminated well among taste cells. The resulting matrix of measurements was analyzed with both hierarchical cluster analysis (SPSS CLUSTER) and multidimensional scaling (SPSS ALSCAL). The multivariate distributions of the cell nuclei were continuous, suggesting that the light/dark cell dichotomy may represent points along a continuum of morphological types.

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651.9

AMILORIDE TREATMENT ELIMINATES THE SOURNESS BUT NOT THE SALTINESS OF SODIUM CHLORIDE AND LITHIUM CHLORIDE. C. A. Ossebaard, K. J. Ciombor, and D. V. Smith*. Dept. Anatomy, Univ. Maryland Sch. Medicine, Baltimore, MD 21201-1559.

Taste receptor transduction of Na^+ salts has been shown in many species to be largely mediated by amiloride-sensitive ion channels on the apical receptor membrane. The residual response to these salts is mediated by a paracellular pathway that is insensitive to amiloride. These biophysical and additional electrophysiological data in rodents have led to the speculation that saltiness arises from this amiloride-sensitive component. We have previously shown that in humans the taste of NaCl is less suppressed by amiloride than the taste of Na-gluconate, which is consistent with the effects of amiloride in the rat. However, amiloride affects only the sour side taste of these salts and produces no effect on their saltiness. This suggests a correlation between stimulation of the apical ion channels and the sourness of Na^+ salts. These apical ion channels have been shown to be more permeable to Li^+ than to Na^+; LiCl also has a larger sour component than NaCl. Therefore, we hypothesized that the taste of LiCl would be more greatly suppressed by amiloride than the taste of NaCl. Human subjects estimated the taste intensity and qualities of several concentrations of NaCl, LiCl, and KCl. Amiloride suppressed the sourness of LiCl more than that of NaCl; there was no effect on the taste of KCl. Estimates of the saltiness of these stimuli were unaffected by amiloride treatment. We propose that Na^+ and Li^+ stimulation of amiloride-sensitive ion channels elicits a sour taste; saltiness may arise via the paracellular pathway.

Supported by NIH grant DC00353 to DVS.

651.10

CHORDA TYMPANI SINGLE-UNIT STUDIES ADDRESSING TASTE CODING OF SODIUM SALTS AND SWEETENERS IN HAMSTERS. B.K Formaker, B.I. MacKinnon, T.P. Hettinger and M.E. Frank*. BioStructure & Function, University of Connecticut Health Ctr., Farmington CT 06030.

We are recording responses of single units in the *chorda tympani* (CT) nerve of golden hamsters (*Mesocricetus auratus*). Stimuli include sodium salts, sweeteners, the saccharide mixture: Polycose, deionized Polycose, and the binary mixture of sucrose plus quinine.HCl (QHCl). Our response measure is the total number of spikes during the initial 5 s of response minus the average spontaneous rate for the unit. Preliminary results show that all tested 0.1 M sodium salts are effective for NaCl-best units. They have the following relative effects: NaCl = NaBr > sodium acetate = sodium benzoate > sodium gluconate. This result demonstrates an effect of anion size on sodium salt transduction in NaCl-best units (Rehnberg *et al.*, 1993). Also, the reliable effect of 10% Polycose on NaCl-best units is eliminated by deionization, indicating that much of the CT response to Polycose is due to its ionic contaminants. The sweeteners 0.04 M sucrose, 0.4 M fructose, and 0.1 M glycine, tested at EC50 concentrations for whole nerve responses (Myers *et al.*, 1993), are all effective stimuli for sucrose-best units. Consistent with the weak behavioral generalization between sucrose and Polycose, deionized Polycose has a very small effect on sucrose-best units. Finally, 0.05 M sucrose is more effective stimulus than sucrose mixed with 0.03 M QHCl for sucrose-best units. This is consistent with the suppression of whole-nerve responses to sucrose by QHCl (Formaker & Frank, 1995). [Supported by NIH R01 DC00058]

651.11

EMERGENCE OF AFFERENT FIBER TERMINAL FIELD ALTERATIONS IN SODIUM RESTRICTED RATS.
B.R. Walker* and D.L. Hill, University of Virginia, Charlottesville, Va 22903

Previous work from our lab has shown that dietary sodium restriction instituted early in prenatal development produces anatomical and functional changes in the developing gustatory system. In particular, neurophysiological recordings from the chorda tympani nerve (CT) demonstrate that whole nerve responses to NaCl are reduced in restricted rats while responses to non-salt stimuli are unaffected. Furthermore, early sodium restriction causes a rearrangement of the CT terminal fields within the nucleus of the solitary tract (NTS) in restricted rats, while glossopharyngeal (IX) terminal fields remain similar to controls. Developmental sodium restriction also produces postsynaptic changes in the NTS. For example, neurophysiological NTS recordings of restricted rats repleted with sodium show hyper-responsive activity to only sodium salts, while Golgi techniques have revealed dendritic alterations of the presumptive projection neurons from the NTS.

To examine the developmental time course of the alterations seen in the NTS in response to the NaCl restriction paradigm, the chorda tympani nerve was labelled in a developmental series with a 3KD biotinylated dextran amine neuronal tracer just peripheral to the geniculate ganglion in sodium restricted and control rats. Results show that the alterations begin at early postnatal time points, and continue through until adulthood. These results suggest that developmental dietary sodium restriction could alter the pathway which the afferent fibers follow to their target.

Supported by the NBD training grant (B.R.W.) and NIH DC 00407.

651.12

MULTIVARIATE ANALYSES REVEAL POTENTIAL RELATIONSHPS BETWEEN THE STRUCTURE AND FUNCTION OF GUSTATORY NEURONS. W.E. Renehan*, Z. Jin, X. Zhang and L. Schweitzer Henry Ford Hospital, Detroit, MI 48202 and Univ. Louisville Sch. Medicine, Louisville, KY 40292.

We have recently shown that a neuron's most effective stimulus ("best response") is a relatively poor predictor of neuronal morphology. The present investigation was conducted to test the hypothesis that individual "best response" groups may be comprised of physiologically-distinct neuronal subsets that do exhibit unique morphologies. Glass micropipettes filled with 2.0% Neurobiotin were used to test the response of NST neurons to 0.1M NaCl (N), 0.01N HCl (H), 0.01M quinine (Q) and 1.0 M sucrose (S). Seventy-one neurons that responded to one or more tastants were injected with Neurobiotin and reconstructed in three dimensions using the Eutectic Neuron Tracing System. Each response was corrected for spontaneous activity and the Pearson correlation coefficients of all possible stimulus-response pairs were calculated. These coefficients were used to perform a cluster analysis (SPSS) and the results of the cluster analysis were used to define physiological subsets. Analyses of variance were then performed to investigate relationships between morphologic features and physiological response properties. We were able to identify a number of trends that appear to deserve further examination with a larger data set. There was a trend ($F=2.2$, d.f.=4,61; $p=0.08$) between mediolateral extent and response category, with a subset of the neurons that responded best to NaCl (N>H>S or Q) more widespread than the S-best or Q-best neurons. This N-best subset was also more broadly tuned than all other groups ($F=4.2$, d.f.=4,61; $p<0.01$) except the Q-best neurons and there was a trend ($F=1.9$, d.f.=4,61; $p=0.12$) that indicated that this group had a larger soma-dendritic receptive field (in the coronal plane) than all other groups. These data indicate that physiologically-defined subsets of the traditional N-, H-, Q- and S- 'best' categories may provide important insights into structure-function relationships in the NST.

Supported by DC01074.

651.13

MORPHOLOGICAL AND FUNCTIONAL PROPERTIES OF ACUTELY ISOLATED NEURONS FROM THE GUSTATORY NUCLEUS OF THE SOLITARY TRACT. R.M. Bradley* and J Du. Dept. Biologic and Materials Sciences, Sch. of Dentistry, Univ. of Michigan, Ann Arbor, MI 48109-1078.

To study the functional properties of identified neurons in the rostral (gustatory) nucleus of the solitary tract (rNST), we have acutely isolated the cells so that direct comparisons can be made between structure and biophysical properties of the neurons. Neurons were isolated from 300 μm thick horizontal brainstem slices from rats aged 6-20 days. The rNST was dissected from the slices, placed in HEPES buffer containing 0.5% protease type 23 and then triturated with a series of different diameter, fire-polished Pasteur pipettes. The dissociated neurons were then placed on a poly-L-lysine coated coverslip in a plastic petri dish and superfused with oxygenated saline. Viewed with an inverted microscope, elongate, multipolar and ovoid neurons could easily be distinguished. These same neuron morphologies have been described in rNST by us and other investigators using a variety of morphological techniques. We recorded from the neurons using the whole cell configuration of the patch clamp technique. The isolated neurons had mean resting membrane potentials of -69 ± 14 mV, a mean input resistance of 308 ± 66 MΩ and overshooting action potentials. When depolarized the neurons either fired a regular train of action potentials or a short burst of action potentials that then accomodated. For some neurons the initiation of the regular train of action potentials was delayed by a brief membrane hyperpolarization. These intrinsic properties are similar to those we have previously reported in whole cell recordings from rNST neurons in brain slices (J.Neurophysiol. 67:1659, 1992). This study shows that neurons acutely isolated from the rNST maintain both their morphological and biophysical properties. (Supported by NIDCD grant DC00288 to R.M.B.)

651.14

CLASSIFICATION OF TERMINALS THAT CONTACT DENDRITIC SWELLINGS ON GUSTATORY NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT (NST). L. Schweitzer, Z. Jin*, T. Cecil, B.M. Wetherton and W.E. Renehan. Univ. of Louisville Sch. Med., Louisville, KY 40292 and Henry Ford Hospital, Detroit, MI 48202.

We have recently established that there are eight morphologically-defined gustatory cells types in the NST. Cells in one of these cell types have small somata (<125 μm^2) and a high density of dendritic swellings (>.05/μm). To investigate the ultrastructural characteristics of terminals that contact the dendritic swellings, cells of this type were processed for electron microscopy. These cells were tested for their responses to an array of tastants, filled with Neurobiotin, reacted with DAB and flat embedded in resin. We were able to verify that dendritic swellings receive synapses. These terminals are of the "primary-like", "SP" and "MP" (small and medium pleomorphic) types as defined by Whitehead (JCN, 332, 1993). We have classified the responses of these cells. A disproportionate number of them are NH4Cl-best and HCl-best. One cell of this type only responded to two (HCl and NH4Cl) of the eight tastants and was NH4Cl-best. This cell receives terminals onto dendritic swellings and the terminals contacting the swellings have rounded or pleomorphic vesicles. We have verified that synapses of all morphological types defined by Whitehead are found in the area in which our gustatory cells are labelled, yet several synaptic types do not appear to contact dendritic swellings.

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651.15

FOREBRAIN PROJECTIONS TO THE ROSTRAL NUCLEUS OF THE SOLITARY TRACT IN THE HAMSTER. M.C. Whitehead*, A. Bergula and K. Holliday. UCSD, La Jolla, CA 92093

The nucleus of the solitary tract (NST) is the first central site of taste information processing. Specific subdivisions of the NST receive taste afferent input and contain interneurons and projection neurons that engage ascending or premotor taste pathways. The forebrain projects to the NST and can influence taste responses, but the anatomical relationship between forebrain inputs, primary inputs and NST cell types is not understood. To evaluate this cholera toxin B (CTb) was injected into the rostral NST to retrogradely label the sources of forebrain inputs. Cells were labelled bilaterally in the hypothalamus, bed nucleus of the stria terminalis, amygdala, and insular (gustatory) cortex. Injections of Ctb into gustatory cortex, the site of most forebrain-NST cells, labelled axon endings confined to the rostral NST. These endings were concentrated in the rostral central and ventral subdivisions. In the rostral central subdivision corticofugal endings are positioned influence microcircuits that include taste afferent synapses, synapses of presumed inhibitory interneurons, and neurons that project to the parabrachial nucleus. In the ventral subdivision corticofugal endings synapse among premotor neurons that ultimately influence salivatory and oromotor outflow.

Supported by NIH grant DC02045

651.16

EXPRESSION OF c-FOS IN THE RAT .PARABRACHIAL NUCLEUS FOLLOWING INGESTION OF MONOSODIUM GLUTAMATE AND OTHER TASTANT STIMULI. S. M. Royer*[1,2], J. C. Kinnamon[1] and S. D. Roper[2]. [1]Dept. of Biological Sciences, Univ. of Denver, Denver, CO 80208 and [2]Dept. of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523.

In rodents, the pontine parabrachial nucleus (PBN) includes the second order gustatory relay in the CNS. In order to compare the localization of PBN responses to monosodium glutamate (MSG) with that of responses to other taste stimuli, we used immunocytochemistry to detect Fos protein expression in PBN neurons following ingestion of tastants. Adult rats were water-deprived and trained to drink during a one-hour period each day. On the experimental day, rats were given either distilled water or a tastant solution during the drinking period. Brains were subsequently fixed by perfusion and the pontine regions sectioned at 50μm on a Vibratome. Fos-like immunoreactivity (Fos-ir) was visualized using a rabbit polyclonal antibody (Santa Cruz Biotechnology, 1:6000). Although sparse Fos-ir appeared throughout the PBN, the location of the greatest concentration of Fos-ir cells occurred in different PBN subnuclei, depending upon the tastant solution ingested. In animals that received distilled water, a distinct cluster of Fos-ir neurons appeared in the dorsal subnucleus (dls) of the lateral PBN. Fos-ir was similarly localized following ingestion of 500mM sucrose. Rats that were given 3mM quinine HCl showed the greatest Fos-ir in the ventral region of the external lateral subnucleus (els) and the external medial subnucleus (ems). In animals that received 500mM MSG plus 30μm amiloride, Fos-ir was concentrated in the els in an area more dorsal than that observed in animals receiving quinine HCl. The region of greatest Fos-ir in response to 500mM MSG appeared to correspond to the zone in which Fos-ir has been observed following intraperitoneal injection of LiCl (Yamamoto et al., NeuroReport 3:1049, 1992).

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651.17

CENTRAL CONSEQUENCES OF GUSTATORY AXOTOMY IN THE RAT: TIME COURSE OF TRANSGANGLIONIC DEGENERATION. M. B. Vogt*, C. Yu, and D. V. Smith. Department of Anatomy, University of Maryland School of Medicine, Baltimore, MD 21201-1559.

Numerous studies have documented the peripheral gustatory system's remarkable capacity for anatomical regeneration and functional recovery following gustatory nerve lesions. However, little is known of the central consequences of gustatory nerve damage. We examined the time course of transganglionic degeneration in the rostral portion of the nucleus of the solitary tract (NST) following combined lesions of the chorda tympani and glossopharyngeal nerves, which innervate taste buds on the anterior and posterior tongue, respectively. In 12 rats a 2-5 mm section of nerve was excised on the right side, distal to the ganglion, from both the lingual-tonsillar branch of the glossopharyngeal nerve and the chorda tympani nerve; animals then survived for 2, 7, 12, 17 or 22 days. Controls included the intact contralateral side in each experimental rat and 3 additional rats that underwent sham surgeries. Brains were sectioned at 40 μm and processed simultaneously with the amino-cupric silver degeneration method. Degenerating fibers were observed ipsilaterally, consistent with the demonstrated projections of these nerves into the rostral NST. Sham-operated and 2-day survival rats showed little or no degeneration. By 7 days there was considerable degeneration which continued to increase through 22 days. Thus peripheral taste nerve section results in transganglionic degeneration which may alter synaptic organization in the NST and provide the opportunity for plasticity during regeneration.

Supported by NIH grants DC02163 to MBV and DC00347 to DVS.

652.1

DOPAMINE INHIBITION OF NUCLEUS ACCUMBENS CELL ACTIVITY OCCURS VIA TWO INDEPENDENT MECHANISMS: EVIDENCE FROM *IN VIVO* AND *IN VITRO* INTRACELLULAR RECORDINGS. A.A. Grace* and P. O'Donnell. *Depts. Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.*

The excitatory or inhibitory nature of dopamine (DA) receptor activation has been a topic of controversy for years. This study analyzes the effects of DA-related drugs on indices of cell excitability *in vitro*, and on the pattern of spontaneous firing observed *in vivo*. Forty-five cells were recorded from slices containing the nucleus accumbens. Perfusion with the D1/D2 agonist apomorphine (10-150 μM) caused the membrane to depolarize from -68.8 ± 8.3 mV to -57.3 ± 10.5 mV without concurrent changes in input resistance or the time constant. The amplitude of current injection required to trigger spike firing was increased from 0.28 ± 0.18 nA to 0.49 ± 0.32 nA ($p<0.005$) following apomorphine, and the membrane potential at which the cells fired was shifted from -44.2 ± 8.2 mV to -33.6 ± 13.1 mV ($p<0.0001$). This decrease in excitability was also obtained with the combined perfusion with a D1 agonist (SKF38393; 3 μM) and a D2 agonist (quinpirole, 10 μM), but not by administration of either drug alone.

Intracellular recordings were made *in vivo* from 2 accumbens cells that exhibited a bistable membrane potential. Systemic administration of a combination of SKF38393 (10 mg/kg, i.v.) and quinpirole (10 mg/kg i.v.) reduced the frequency at which the membrane switched to the depolarized, active state from 1 Hz to 0.5 Hz in both cells tested. Since we have previously shown that responses to prefrontal cortical inputs can be elicited only during the depolarized state (J.Neurosci,1995,15:3622-3639), a decrease in the frequency of depolarizing periods would result in a functional blockade of cortical throughput in the nucleus accumbens. Furthermore, both *in vivo* and *in vitro* actions appear to be mediated by coactivation of D1 and D2 receptors. Thus, despite the DA-mediated depolarization of accumbens cell membranes, the combined effects of a decrease in cell excitability and the reduction in transitions to the depolarized state would independently produce an inhibitory influence on nucleus accumbens neuron activity. Supported by MH42217, 45156, 01055 (AAG), and Tourette's Syndrome Association (P.O'D.).

652.2

THE VENTRAL PALLIDUM → ACCUMBENS FEEDBACK PATHWAY: ANATOMICAL AND ELECTROPHYSIOLOGICAL STUDIES.

M. Wu, A. W. Hrycyshyn S. M. Brudzynski*. Dept. of Physiology and Dept. of Anatomy, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

Earlier evidence has suggested that a feedback loop may exist from the nucleus accumbens to the ventral pallidum (VP), to the ventral tegmental area (VTA), and back to the nucleus accumbens. Recent anatomical studies have also shown that a reciprocal projection between VP and the accumbens exists. In the present study, anatomical and electrophysiological techniques were used to investigate the functional organization of these feedback loops.

The anterograde tracer dextran conjugated rhodamine was injected into the VP of rats. Labelled terminals were found in both the nucleus accumbens and the VTA. In standard extracellular recording studies, electrical stimulation of the VP evoked mostly inhibitory responses of the accumbens and VTA neurons. 75% of the responded neurons had a latency of less than 10 ms. Furthermore, the injection of the glutamate into the VP not only altered the firing pattern of the accumbens neurons but also attenuated the excitatory responses of these neurons elicited by electrical stimulation of the hippocampus.

Since the feedback loop of VP→VTA→accumbens would likely take a longer time to complete than the more direct loop of VP→accumbens, the results from present study suggest that a direct VP→accumbens pathway may influence the limbic input to the nucleus accumbens. (Supported by MRC of Canada)

652.3

IMPAIRED ACQUISITION OF INTRACRANIAL SELF-STIMULATION IN RATS WITH BILATERAL KAINIC ACID-INDUCED LESIONS OF NUCLEUS ACCUMBENS SEPTI. A.Yu.Bespalov* and E.E.Zvartau. Dept. of Pharmacology, Pavlov Med. Univ., St.-Petersburg 197089, Russia.

Excitotoxic lesions of nucleus accumbens septi (NAS) were previously shown to have a delayed effect on the intracranial self-stimulation (ICSS) responding in rats. The present study addressed the ability of kainic acid-induced lesions of NAS to affect acquisition of ICSS. Male Wistar rats were implanted with a unilateral bipolar electrode into the ventral tegmental area and bilateral cannulas were implanted into the NAS. Seven days postoperatively two groups of animals - lesioned and sham-lesioned - were trained to respond for the response contingent electrical stimulation (continuous reinforcement schedule). Fourteen 28-min training sessions were performed once a day by an experimenter blind to the grouping factor. Percent of animals responding for electrical stimulation by the end of the training period were calculated after histological verification of cannula/electrode placement and evaluation of the size of lesions. The kainic acid-induced damage was generally restricted to the NAS and minor damage to caudatus-putamen was consistently present. Only 2 out of 10 kainic acid-lesioned animals responded more than 200 times a session by the last training session, whereas 5 out 6 control sham-lesioned successfully acquired the SS responding ($p<0.05$, chi-square test). These data suggest that NAS is necessary for the acquisition of ICSS. (Supported in part by grant NWO 07-13-087 of Dutch Medical Council).

652.4

REGULATION OF PREPULSE INHIBITION IN THE RAT BY VENTRAL PALLIDAL PROJECTIONS. M.H. Kodsi*, N. Taaid, H.I. Hartston, D. Zisook, F.I. Wan, N.R. Swerdlow. UCSD Dept. Neurosci., La Jolla, CA 92093.

Prepulse inhibition (PPI) of the acoustic startle reflex is the reduction of startle amplitude by weak prestimuli delivered 30-500 msec prior to the startling stimulus. Since PPI is significantly reduced in specific neuropsychiatric disorders, studies have attempted to elucidate the neural substrates that regulate PPI. Previous observations have shown that PPI is regulated by GABAergic projections from the ventral striatum to the ventral pallidum (VP). The GABA-A antagonist picrotoxin activates VP neurons and dose-dependently reduces PPI. In order to determine which VP projections carry modulatory signals to the primary startle circuit, we measured PPI after quinolinic acid lesions of the subthalamic nucleus (STN), mediodorsal thalamus (MD), and pedunculopontine tegmental nucleus (PPTg). We also determined the effects of these lesions on apomorphine reduction of PPI and on amphetamine-stimulated locomotion. Since VP efferents include GABAergic fibers, we also measured PPI after infusion of the GABA-A agonist, muscimol, into relevant VP targets. Of the three excitotoxic lesion sites, only PPTg damage significantly reduced PPI, and only STN lesions significantly disrupted amphetamine-stimulated locomotion. Apomorphine-induced reduction of PPI was not significantly altered by any of the lesions. Muscimol infusion into the PPTg dose-dependently reduced PPI. PPI thus appears to be regulated by ventral pallidal projections to the PPTg, but not to the STN or to the MD. Furthermore, activation of PPTg GABA-A receptors mimics the PPI disruption that follows activation of the VP. These findings collectively support the notion that PPI is regulated by a GABAergic projection from the VP to the PPTg.

652.5

CORTICO-STRIATAL SUBSTRATES OF PREPULSE INHIBITION OF ACOUSTIC STARTLE IN THE RAT F.I. Wan*, M.H. Kodsi, S.B. Caine and N.R. Swerdlow. Depts. of Neuroscience and Psychiatry, Univ. of California San Diego, La Jolla, CA 92037-0804.

Inhibition of the acoustic startle reflex by a weak "prepulse" is an operational measure of sensorimotor gating that can be studied in both humans and rats. Since deficits in prepulse inhibition (PPI) are characteristic of several neuropsychiatric disorders including schizophrenia, it is of interest to elucidate the neural substrates underlying PPI. Importantly, recent studies using neuroimaging and neuropathological methods have identified abnormalities within limbic cortico-striatal circuitries in schizophrenia patients. In the present experiment, we examine limbic cortico-striatal substrates of PPI in the rat. Preliminary data shows that PPI is disrupted after infusion of the non-NMDA agonist AMPA into the nucleus accumbens (NAc) core and shell subregions. This effect of AMPA in the core can be blocked by systemic pretreatment with the D2 antagonist haloperidol (0.1mg/kg), whereas that of AMPA in the shell appears not to be blocked by haloperidol. Infusion of NMDA (0.8μg) into the ventral subiculum of the hippocampus significantly reduces PPI. This effect of NMDA on PPI is not prevented by the non-NMDA antagonist CNQX (2.5μg) infusion into the NAc core or shell subregion, or by systemic injection of haloperidol. PPI is reduced after infusion of either NMDA or the NMDA antagonist AP5 into the basolateral amygdala, but not after quinolinic acid (0.15M/0.3μl) lesions of the basolateral amygdala. We are now studying the potential contribution of the prefrontal cortex and its connection with the NAc to the modulation of PPI. Our results suggest that glutamatergic limbic cortico-ventral striatal circuitries play an important and complex role in the regulation of sensorimotor gating in the rat.

652.6

DOPAMINE RECEPTOR MODULATION OF WHOLE CELL SODIUM CURRENT IN ACUTELY DISSOCIATED RAT NUCLEUS ACCUMBENS NEURONS. X.-F. Zhang* and F. J. White Dept. Of Neuroscience, FUHS/ The Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064-3095

The nucleus accumbens (NAc) is a ventral striatal region that serves as an interface between limbic and motor systems and thereby plays essential roles in motivational aspects of behavior. Therefore, NAc neurons and their dopaminergic (DA) innervation have become the subject of considerable interest and their physiological properties are currently being studied under a variety of conditions. In the present studies, we used intracellular current clamp and whole-cell voltage clamp techniques to investigate dopamine receptor-mediated modulation of TTX-sensitive sodium current (I_{Na}). In adult rat NAc slices, bath application of DA (25 μM, n=6) significantly hyperpolarized NAc neurons, increased the amount of intracellular current injection required to generate action potentials, increased the spike threshold and decreased spike amplitude. Similar results were observed in two neurons with application of the DA D_1 receptor-class agonist SKF 38393. For whole-cell recordings, NAc neurons from 1-4 weeks old rats were acutely dissociated. Only medium-sized neurons (6-12 μm) were used. Application of DA (10-25 μM) reduced peak I_{Na} (3/3). SKF 38393 (1-5 μM) also reduced peak I_{Na} (10/12), an effect that was completely blocked by the DA D_1-class receptor antagonist SCH 23390 (5 μM). The degree of inhibition produced by SKF 38393 was 6.5-30.8%. This agonist reduced peak I_{Na} without affecting the activation pattern. Instead, the steady-state inactivation curve was shifted about 5 mV in the negative direction. The DA D_2-class receptor agonist quinpirole (1-5 μM) exerted diverse effects on I_{Na}, in that two (of 8) neurons responded with increased current, whereas four responded with decreased I_{Na}. Both of the effects were completely blocked by DA D_2-class receptor antagonist eticlopride (5 μM). These results indicate that DA D_1 receptor activation reduces I_{Na}, results in hyperpolarization of NAc neurons and decreased excitability. The effects of DA D_2-class receptor activation appear more complex and variable, perhaps indicating multiple receptor involvement. (Supported by DA04093 and DA00207 to FJW).

652.7

CHOLECYSTOKININ-MEDIATED INCREASES IN AXONAL TERMINAL EXCITABILITY OF DOPAMINERGIC AFFERENTS TO ACCUMBENS. M. Garcia-Munoz*, P. Patino, S.J. Young and P.M. Groves. Department of Psychiatry, School of Medicine, University of California, San Diego. La Jolla, CA 92093-0603.

The nucleus accumbens (NA) receives afferents that contain both dopamine (DA) and cholecystokinin (CCK). It has been proposed that CCK can modify the release of DA by acting on presynaptic autoreceptors located on DA-CCK afferents. Electrical excitability testing of terminal axons was employed to study the consequences of increased impulse flow in DA-NA afferents on presynaptic effects of CCK in urethane anesthetized rats. Excitability was measured as the probability of antidromic firing of DA neurons after stimulation of NA. Throughout the experiment, the stimulus current was maintained at a constant value, determined during the baseline period, that elicited antidromic responses on approximately 50% of the stimulus presentations. To increase impulse activity, the medial forebrain bundle (MFB) was stimulated with three pulses at 20 Hz, 100ms prior to the NA test stimulus. This sequence was repeated at 1 Hz for 5 min. The increase in impulse activity resulted in an enhancement in terminal excitability. Intra-NA infusion of the CCK-A or CCK-B receptor antagonists devazepide (10nM) or L-365-260 (20nM), respectively, just prior to the MFB conditioning trains prevented the stimulus-induced increase in excitability. Instead, MFB stimulation produced a decrease in DA-NA afferent terminal excitability. This decrease in excitability was abolished by impairing DA transmission with α-methyl-*p*-tyrosine (250 mg/kg, i.p., 12 and 2 h before recording). We conclude that during higher rates of impulse activity, CCK released from DA-CCK containing afferents can stimulate presynaptic receptors to increase excitability and DA release.

652.8

DISTINCTIVE ROLES OF GLUTAMATE RECEPTOR SUBTYPES OF THE STRIATUM *IN MOVEMENT* SELECTION: A HYPOTHESIS FOR SERIAL ORGANIZATION. M. Pisa*. Dept. Biomed. Sci., McMaster Univ., Hamilton, Ont., Canada, L8N 3Z5.

In support of the hypothesis of distinctive motor roles of NMDA and AMPA receptors of the striatum, we previously reported that blockade of NMDA receptors of the ventrolateral striatum (VLS) of male Wistar rats by injections of CPP (1-10 nmole) significantly increased chewing in vacuo, a nonexternally directed oral movement, while decreasing grooming, an externally directed oral movement toward the animal's own fur. In contrast, blockade of AMPA receptors by injections of NBQX (1-10 nmole) into the VLS significantly reduced grooming, but did not increase chewing in vacuo. The trend for NBQX actually to reduce chewing in vacuo was not statistically significant, apparently owing to a floor effect, i.e. a low baseline level of chewing in vacuo in vehicle treated animals. The hypothesis that AMPA-receptor blockade depresses both directed and nondirected oral movements was tested by examining the motor effects of NBQX injections (1-10 nmole/0.5 μL) into the VLS against a background high level of chewing in vacuo induced by CPP injections (5 nmole/0.5 μL) into the VLS. Injections of CPP alone replicated the motor effects found previously. Co-injections of NBQX with CPP virtually abolished the high levels of chewing in vacuo induced by CPP alone. The co-injections also significantly reduced grooming relative to vehicle controls, but not compared with CPP alone, owing to a ceiling depressant effect of CPP on grooming. These results are consistent with the hypothesis that AMPA and NMDA receptors of the VLS play distinctive roles in the initiation of reaching and chewing components of appetitive-consummatory oral motor sequences. More specifically, I propose that co-activation of both receptor subtypes mediates the initiation of oral reaching, whereas hypoactivity specifically of NMDA receptors mediates the initiation of (transition to) chewing. *Hypoactivity of AMPA receptors suppresses all movements.* (Supported by NSERC. M. Pisa is a Research Associate of the Ontario Mental Health Foundation).

BASAL GANGLIA: NIGRA AND RELATED SYSTEMS

653.1

MODULATION OF SUBSTANTIA NIGRA DOPAMINE NEURON ACTIVITY BY MUSCARINIC ANTAGONIST ADMINISTRATION. C.L. Todd, W. Cameron* and A.A. Grace. Depts. of Neuroscience and Psychiatry, Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Benztropine (Benz) is a muscarinic antagonist that is used clinically to treat acute dystonia, an extrapyramidal side effect induced by antipsychotic drugs (APDs). Acute dystonia typically occurs within the first four days of treatment with APDs, and is more common when high-potency APDs (e.g. Haloperidol (HAL)) are used. Although the effects of APDs on the firing rate of substantia nigra (SN) DA cells have been well characterized, little data regarding the effects of Benz on these cells has been reported. Therefore, in this study we examined the effects of Benz on SN DA cell firing using *in vivo* extracellular single unit recordings from male Sprague-Dawley rats anesthetized with chloral hydrate, 400 mg/kg, i.p. Recordings of baseline activity were made from identified DA cells for at least five minutes prior to and following administration of each drug. In one group of animals, the effects of Benz administered alone was tested. Benz administered in a dose-response manner (initial dose 0.01 mg/kg i.v.) caused a significant dose-dependent decrease in mean firing rate in 4/5 cells tested. In this group, the mean cumulative dose of Benz required to cause a decrease in firing rate of 10% was 0.55 ± 0.30 mg/kg. In another group of rats, the effects of HAL pretreatment on the response to Benz was tested. Hal administration resulted in a significant increase in firing rate in 4/5 DA cells tested. Subsequent administration of Benz also caused a significant dose-dependent decrease in firing rate in 4/5 cells tested. The mean cumulative dose of Benz required to cause a 10% decrease in firing rate from that present after HAL was 0.06 ± 0.04 mg/kg. In a third group of rats, Benz was given in a single bolus dose of 15 mg/kg i.v., either with (n=2) or without (n=1) previous treatment with Hal (0.1 mg/kg i.v.). In all three cases, Benz caused a 100 % inhibition of firing. Therefore DA cells were found to be more sensitive to Benz-induced inhibition following HAL pretreatment. This interaction may account for the effects of Benz in treating neuroleptic-induced acute dystonia in humans. Supported by MH42217, MH01055, NS19608.

653.2

HIPPOCAMPAL ACTIVATION SUPPRESSES VTA DOPAMINE CELL FIRING. A POTENTIAL ROLE FOR HIPPOCAMPAL REGULATION OF PHASIC DA RELEASE. D.G. Harden* and A.A. Grace. Depts. Of Neuroscience and Psychiatry, Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Post-mortem studies of the brains of schizophrenics suggest that hippocampal damage may play an important role in the etiology of this disorder. Although several studies have examined the impact of hippocampal lesion on dopamine (DA) function in striatal targets, the influence of hippocampal activity on DA cell firing has not been reported. This study examines the effects of hippocampal activation on VTA DA cells recorded in rats. In one set of experiments, electrical stimulation consisting of single pulses with a duration of 0.5 msec and intensity ranging from 0.5-1.0 mA were delivered to the fornix. Activation of the fornix potently inhibited 27/31 (87%) of VTA DA cells tested. The mean onset latency of the inhibition was 33.0 ± 4.2 msec (1◡-90 msec) and the mean duration was 198.9 ± 28.3 msec (40-515 msec). To address the possibility that the observed suppression was due to inadvertent activation of non-hippocampal projections, direct stimulation of the CA1/subicular region was performed in a second set of experiments. Similar to fornix stimulation, direct activation of the hippocampus also resulted in inhibition of VTA DA cell firing in 15/32 (47%) of the neurons examined. The mean onset latency of the response was 44.7 ± 6.5 msec (10-95 msec) and the mean duration was 127.8 ± 49.5 msec (35-705 msec). Because there are no direct projections from the hippocampus to the DA cells of the VTA, the observed inhibition is likely to be mediated by polysynaptic pathways involving the nucleus accumbens or prefrontal cortex or both.

The finding that hippocampal activation suppresses DA cell firing, suggests that hippocampal damage reported to be present in schizophrenia could potentially disrupt subcortical DA systems by reducing an inhibitory influence at the level of the cell body. This work was supported by NS19608, MH42217, MH 01055.

653.3

ACTIONS OF SEROTONIN ON SUBSTANTIA NIGRA PARS RETICULATA NEURONS IN VITRO. I.M. Stanford* and M.G. Lacey. Dept of Pharmacology, Univ. of Birmingham, Birmingham, B15 2TT. U.K.

The GABA containing neurons of the substantia nigra pars reticulata (SNr) constitute a major relay of basal ganglia output projecting to the thalamus, superior colliculus and brainstem. The SNr appears to have a role in controlling eye movement, promoting voluntary movement and has been implicated in the generation of seizure activity. SNr neurons receive inputs from GABA-containing pallidal and striatal neurons, glutamate-containing subthalamic nucleus neurons as well as a serotonin input from the dosal raphe. Whole-cell patch clamp recordings were made from SNr neurons from 300μm parasaggital slices of midbrain from rats 9-12 days of age. Individual neurons were visualized and recordings made using a Nomarski optical system (Zeiss Axioskop, x40 water immersion objective). SNr neurons were easily identified as they fired spontaneous action potentials at a fast rate (range 5-38Hz, mean 15.6 ± 7.9Hz, n=39), showed no evidence of I_h and were insensitive to quinpirole (10μM) and met-enkephalin (10μM), pharmacologically distinguishing them from dopamine cells and interneurons of the pars compacta. Serotonin (10μM) caused an inward current accompanied by an increase in membrane conductance (5/5 cells). The inward current or depolarization was insensitive to TTX (1μM, n=2) indicating a direct action on SNr neurons, possibly through action at a 5-HT_{2C} receptor (Rick & Lacey, B.J.Pharm. 112, 490P, 1994). Single shock stimulation of the descending inputs, in the presence of CNQX (10μM) and D-APV (50μM) elicited a picrotoxin sensitive IPSC which reversed around E_{Cl}. Serotonin (3-10μM) reduced this evoked IPSC (7/7 cells) whereas spontaneous GABA IPSCs (sIPSCs) were enhanced (3/3 cells). Evoked and sIPSCs were sensitive to TTX (1μM). The increase in frequency of sIPSCs may be a direct consequence of the SNr neuron depolarization and increased GABA release from axon collaterals, while depression of the evoked IPSC may be due to the presynaptic modulation by 5-HT receptors found on striato-nigral terminals.
(Supported by the Wellcome Trust)

653.4

INHIBITION BY METABOTROPIC GLUTAMATE RECEPTORS OF EXCITATORY SYNAPTIC INPUT TO RAT MIDBRAIN DOPAMINE NEURONS *IN VITRO*. M.A. Wigmore and M.G. Lacey*. Dept of Pharmacology, Univ. of Birmingham, B15 2TT. U.K.

Dopamine plays a critical role in the control of voluntary movement and is released within the basal ganglia from the terminals of dopamine-containing substantia nigra pars compacta (SNc) dopamine cells in the midbrain. One likely source of excitatory drive to the SNc is the glutamate-containing projection from the subthalamic nucleus (SThN). Descending synaptic input to SNc has been examined here using intracellular microelectrode recording from SNc neurones in 400 μm thick parasagittal slices of rat midbrain, maintained at 33-34°C and superfused with a standard medium. SNc dopamine neurons were recognised by pacemaker firing of action potentials at up to 6 Hz and a large I_h current on hyperpolarization under voltage clamp.

Synaptic potentials were evoked by stimulating a region 200-500 μm rostral to the substantia nigra using a focal bipolar stimulating electrode, while holding membrane potential at between -70 and -80 mV with constant hyperpolarizing current (10-30 pA). In the presence of picrotoxin (50 μM) to block $GABA_A$ receptors, the residual synaptic potentials (EPSPs) were blocked by CNQX and APV, indicating that they were glutamate mediated. The broad spectrum metabotropic glutamate receptor (mGluR) agonist 1-aminocyclopentane-1S,3R-dicarboxylate (1S,3R-ACPD; 30 μM) caused a reversible depression of 38 ± 4 % of this glutamate receptor- mediated EPSP (6/6 cells). A reversible membrane depolarisation of 3 - 10 mV was also observed. Both the depression of the EPSP and the depolarization caused by ACPD was reversed by the mGluR antagonist α-methyl-4-carboxyphenylglycine (MCPG; 500 μM; 3/3 cells).

These data suggest that excitatory afferents to dopamine cells possess mGluR receptors that act to reduce glutamate release from the terminals.

MAW is a Wellcome Prize Student

653.5

ELECTROPHYSIOLOGY OF ANTISENSE KNOCKOUT OF D_2 AND D_3 DOPAMINE RECEPTORS IN NIGROSTRIATAL DOPAMINE NEURONS. B.-C. Sun*, Ian Creese, and J.M. Tepper. Center for Molecular & Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

We have shown that infusion into substantia nigra of a 19-mer antisense (AS) oligodeoxynucleotide corresponding to codons 2-8 of the mRNA for the dopamine (DA) D_2 receptor for 6 days greatly attenuates apomorphine-induced inhibition of firing of nigral DA neurons (*Soc. Neurosci. Abstr. 20*:908, 1994).

Very similar effects were obtained following treatment with a D_3 receptor AS. Treatment with D_2 and D_3 AS appeared to be additive, suggesting a role for both D_2 and D_3 receptors in the inhibition of firing of DA neurons in response to DA agonists. Three days of AS infusion produced effects similar to those after 6 days, suggesting a relatively rapid rate of DA receptor turnover and transport. Only D_2 AS knockout increased spontaneous firing rates ($F=5.1$, $df=3,96$, $p<0.01$); Control (C): 3.4±0.3 spikes/s, D_2 AS: 4.7±0.5, D_3 AS: 3.3±0.3, D_2+D_3 AS: 2.7±0.4. Measurements of the proportion of striatal-evoked antidromic responses consisting of the full spike, an index of somatodendritic excitability, revealed a significant increase in full spike responses in all groups: C: 17.9±3.6%, D_2 AS: 41.1±7%, D_3 AS: 37.3±7.1%, D_2+D_3 AS: 45.0+9.1%. Terminal excitability as measured by antidromic threshold currents was increased following AS treatment ($F=2.9$, $df=3,60$, $p<0.05$); C: 1.7±0.3 mA, D_2 AS: 1.1±0.17 mA, D_3 AS: 1.0±0.2mA, D_2+D_3 AS: 0.9±0.2 mA.

These data suggest that functional D_2 and D_3 autoreceptors are present at the somatodendritic and terminal regions of nigrostriatal neurons. Differences in the effects of D_2 and D_3 AS knockout on spontaneous firing suggest that D_2 receptors may be preferentially located relatively close to the spike-generating region of the neuron while D_3 receptors may located more distally where they modulate local dendritic excitability without affecting overall firing rate. Supported by MH52383, and Rutgers grants from Hoechst-Celanese, and Johnson & Johnson.

653.6

DIFFERENTIAL GABA RECEPTOR MEDIATED INHIBITION OF SUBSTANTIA NIGRA DOPAMINERGIC NEURONS FROM NEOSTRIATUM AND GLOBUS PALLIDUS C. A. Paladini*, P. Celada, T. M. Tran, and J. M. Tepper. Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ USA 07102

Substantia nigra (SN) pars compacta dopaminergic (DA) neurons are inhibited by pars reticulata axon collaterals acting exclusively at $GABA_A$ receptors (Tepper et al., *J. Neurosci. 15*:3092-3103, 1995). The receptors mediating the inhibition of SN DA neurons from globus pallidus (GP) and striatum have been inferred to be $GABA_B$ and $GABA_A$ respectively, but this remains to be conclusively demonstrated.

Adult male Sprague-Dawley rats were anesthetized with urethane and bipolar stimulating electrodes were implanted in the anterior-lateral striatum and GP. Single unit responses following stimulation of striatum and GP (0.5 - 2.0 mA, 0.25 - 0.5 ms duration at 0.67 Hz) were recorded extracellularly in SN pars compacta and the effects of systemic application of the $GABA_A$ antagonist, bicuculline, (0.2 - 1.0 mg/kg i.v.), on evoked responses were examined. All responses were analyzed using peristimulus time histograms and cumulative sum histograms.

Striatal stimulation inhibited SN DA neuron firing by an average of 69.6% in all of the neurons selected ($n = 7$) for bicuculline administration for a duration of 95.2 ± 29.7 ms (mean ± SEM). Stimulation of GP ($n = 11$) produced a 76.9% inhibition for a duration of 30.2 ± 12.5 ms. The onset latencies for inhibition from both GP (2.3 ± 0.9 ms) and striatum (11.4 ± 1.7 ms) were consistent with values previously reported for monosynaptic responses in SN evoked from these nuclei. After administration of bicuculline, striatal-evoked inhibition was significantly blocked or attenuated ($t = 3.94$, $p < 0.01$), but GP-evoked inhibition was not affected (72.1% inhibition). These data suggest that monosynaptic inhibition of SN DA neurons from striatal stimulation, but not from GP stimulation, is $GABA_A$ receptor mediated and support the previous suggestion that GP-evoked inhibition of SN DA neurons may be $GABA_B$ receptor mediated. Supported by MH45286 and NS30679. P. Celada was supported through a Spanish Government Fellowship.

653.7

INWARD AND OUTWARD RECTIFICATION IN NEOSTRIATAL NEURONS DURING THE EARLY POSTNATAL PERIOD. T. Kogs, F. Trent* & J.M. Tepper. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Previously, we have shown that about 1/3 of rat striatal medium spiny neurons *in vivo* exhibit a slowly developing outward rectification (OR) at strongly hyperpolarized membrane potentials and a fast outward rectification in the depolarizing direction during the first two postnatal weeks (Tepper and Trent, *Prog. Brain Res. 99*:35, 1993) with the remainder exhibiting relatively linear I-V curves. In contrast, adult medium spiny neuron I-V curves are dominated by a fast inward rectification.

400 µm neostriatal slices from Sprague-Dawley rats (3 to 11 days of age) were cut in a modified horizontal plane. Intracellular recordings were obtained from submerged slices at 32° C with glass micropipettes filled with 1.5 M KAc and 3-8% neurobiotin possessing 60-100 MΩ resistance.

Hyperpolarizing current pulses (150-300 ms) revealed a modest OR that became very pronounced at membrane potentials negative to -110 mV, resulting in a hyperpolarizing ramp. After application of 2 mM Cs^+ the activation range for the ramp potential and the OR shifted in the positive direction and could be initiated at potentials more negative than -80 mV. 10 mM TEA or 300 µM Ba^+ (with or without Cs^+) blocked the ramp potential and the OR and linearized the neuron's response to hyperpolarizing current pulses. These drugs also increased the input resistance approximately to the value measured at the end of the most hyperpolarized potentials in the absence of any channel blockers (200-250 MΩ). In some neurons 2-10 mM TEA revealed a Cs^+ sensitive time dependent inward rectification. The outward rectification in the depolarizing direction could be blocked by 3 mM, but not 300 µM Ba^+.

These data suggest that the hyperpolarizing ramp is due to the slow deactivation of a TEA and Ba^+-sensitive potassium conductance. We suggest that the time course of opening of the Cs^+-sensitive inward rectifier channel and the closing of the TEA/Ba^+-sensitive outward rectifier are similar and their sum remains constant over time and linearizes the neuron in the negative voltage range close to rest. Supported by NS30679.

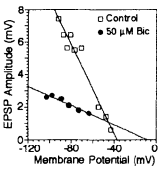
653.8

SUBTHALAMIC STIMULATION-INDUCED SYNAPTIC RESPONSES IN NIGRAL DOPAMINERGIC NEURONS *IN VITRO* K. Moore, K. Pang & J.M. Tepper* Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ USA 07102

Although the efferent projections from the subthalamic nucleus (STN) contain an excitatory amino acid, dopaminergic (DA) substantia nigra neurons *in vivo* usually respond to STN stimulation with an initial inhibition of firing (Smith and Grace, *Synapse 12*:287, 1992). Since 90% of STN-nigral fibers terminate on non-DA neurons, activation of which inhibits DA neurons via $GABA_A$ synapses (Tepper et al., *J. Neurosci. 15*:3092, 1995), we hypothesized that the STN-evoked inhibition of DA neurons arises through activation of pars reticulata axon collaterals.

Parasagittal 400 µm slices containing STN and substantia nigra were prepared from adult male Sprague-Dawley rats. Intracellular recordings were obtained from submerged slices at 32° C with glass micropipettes filled with 1.5 M KAc and 3% biocytin possessing 60-100 MΩ resistance.

Stimulation of STN produced depolarizing responses in electrophysiologically identified nigral DA neurons (n=5, max. amplitude=6.3±1.6 mV at rest; E_m = -69.2±6.7mV, onset latency=2.9±0.3ms, duration= 26.9 ±3.2ms). These responses appeared to be depolarizing synaptic responses since they increased with membrane hyperpolarization and decreased with membrane depolarization (see Figure). However, the response had an unusually negative reversal potential of -35 to -40 mV. Bath application of the GABAA antagonist, bicuculline (50 µM), shifted the reversal potential to -5 to -10 mV.



We suggest that STN stimulation produces a near simultaneous EPSP and IPSP in DA neurons. The EPSP appears to be monosynaptic, from STN, and the IPSP disynaptic, mediated through $GABA_A$ receptors activated by the axon collaterals of pars reticulata GABAergic neurons. Supported by MH-45286 and NS30679.

653.9

STIMULATION OF STRIATAL D1 AND D2 RECEPTORS IS NECESSARY BUT NOT SUFFICIENT FOR SYNERGISTIC EFFECTS OF SYSTEMICALLY ADMINISTERED DOPAMINE AGONISTS ON SUBSTANTIA NIGRA PARS RETICULATA NEURONS. **M. J. Twery*, D. A. Bergstrom and J. R. Walters. ETB, NINDS, Bethesda, MD 20892.**

Concurrent intravenous (i.v.) administration of the dopamine D1 agonist SKF 38393 and the D2/D3 agonist quinpirole inhibits the activity of substantia nigra pars reticulata (SNpr) neurons in rats with 6-hydroxydopamine (6OHDA)-induced lesions of midbrain dopamine cells. In order to investigate the role of striatal D1 and D2 receptors, agonists were administered locally in striatum while the activity of individual SNpr neurons was recorded extracellularly in gallamine-paralyzed rats with 6OHDA lesions. Striatal infusion (0.5 µl/10 min) of either SKF 38393 (17 nmols, n=14), quinpirole (19 nmols, n=9), or the combination of both agonists (n=6) produced no change in SNpr firing rate. However, striatal infusion of quinpirole and i.v. SKF 38393 (3.4 mg/kg) decreased SNpr neuronal activity to 49 ± 19% (n=10) of baseline. The i.v. SKF 38393 alone produced no change in firing rate. Striatal infusion of the D1 antagonist SCH 23390 (6 nmols) blocked the inhibitory response to concurrent i.v. administration of SKF 38393 and quinpirole (0.3 mg/kg) in 7 of 15 neurons. These results indicate a requirement for concurrent stimulation of striatal D1 and D2 receptors in the mechanism underlying synergistic inhibitory effects of i.v. dopamine agonists on SNpr neuronal activity. However, local stimulation of striatal dopamine receptors did not inhibit SNpr neurons. The findings suggest that the inhibitory mechanism can be activated if additional D1 sites are stimulated as was evident with i.v. SKF 38393. Systemically administered dopamine agonists acting principally at D1 and D2 receptors in striatum and D1 receptors at other sites could decrease SNpr neuronal activity either through convergent pathways or through multiple sites associated with a single pathway.

653.10

SIMULATION OF THE INTRINSIC MEMBRANE PROPERTIES OF GUINEA-PIG AND RAT MIDBRAIN DOPAMINE NEURONE. W.H.Yung. (SPON: The Hong Kong Society of Neurosciences). Department of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong

A biophysical model capable of exhibiting some of the characteristic properties of midbrain dopamine neurones of the guinea-pig and rat is described and simulated in the computer. The active membrane properties are reconstructed by simulating the following ionic conductances: a fast sodium conductance, a delayed potassium conductance, a hyperpolarisation-activated potassium conductance, a fast transient outward potassium conductance, a low threshold calcium conductance, a high threshold calcium conductance and a slow calcium-activated potassium conductance (gKCa).

The kinetic parameters of these ionic channels were obtained from voltage-clamp data of both dopamine and non-dopamine neurones. The model is capable of exhibiting the following characteristics of dopamine cells: slow pace-maker firing, a prominent slow after-hyperpolarisation (AHP) and a strong inward rectification.

A critical component of the model is the modelling of the slow AHP by combining a simple first-order calcium buffering model and a three-state kinetic model of the gKCa. Calcium influx through the high-threshold calcium channel activates the gKCa. The time course of the AHP is determined by the kinetics of the gKCa rather than the intracellular calcium concentration.

653.11

HISTAMINE H_3-RECEPTOR ACTIVATION INHIBITS K+-EVOKED GABA RELEASE IN RAT SUBSTANTIA NIGRA PARS RETICULATA SLICES. J.A. Arias-Montaño[1]*, M. García[1], J. Aceves[1], B. Florán[1], and J.M. Young[2], [1]Departamento de Neurociencias, CINVESTAV-IPN., México, D.F., México and [2]Department of Pharmacology, University of Cambridge, England.

The substantia nigra pars reticulata (SNr) plays an important role in the regulation of basal ganglia motor functions. The spontaneous activity of SNr neurones is controlled by striatal GABAergic projections and by dopamine (DA) released from dendrites of neurones located in the substantia nigra pars compacta (SNc). There is evidence that histamine is also involved in the control of locomotor activity and high levels of H_3-receptors have been found in SNr (Pollard *et al.*, Neuroscience, 52:169-189, 1993). Therefore we set out to study whether H_3-receptor activation modulates GABA or dopamine release in SNr. Coronal slices (300 μm thick) from SNr were labelled in Krebs-Henseleit (K-H) buffer with either [^{3}H]-GABA (glial uptake prevented by 10 μM β-alanine) or [^{3}H]-DA. After washing out the excess of radioactivity, slices (6-8 per chamber) were perfused with KH buffer (1 ml min^{-1}) and perfusates (collected every 4 min) were analysed by liquid scintillation spectrometry. High K+ (15 mM)-induced depolarisation resulted in increased [3H]-GABA efflux (2.8 ± 0.2 fold of basal release) and [3H]-DA efflux (1.83 ± 0.08 fold of basal), as estimated at the peak of tritium release. The selective H_3 agonist immepip (1 μM) decreased (by 53 ± 8%) K+-evoked [3H]-GABA release. This effect was fully reverted by thioperamide (1 μM), a selective H_3 antagonist. Depolarisation-induced [3H]-DA release was slightly reduced by immepip, but the inhibition failed to yield statistical significance. These results suggest that histamine may regulate SNr function by inhibiting GABA release via H_3-receptors mainly located on striatonigral terminals.

MUSCLE: CELLULAR AND MOLECULAR PHYSIOLOGY

654.1

ALTERED MYOSIN mRNA AND PROTEIN CONTENT IN RAT SOLEUS AND TIBIALIS ANTERIOR MUSCLE FOLLOWING FOREIGN REINNERVATION. K.A. Huey and S.C. Bodine-Fowler*. Dept Orthopaedics and Biomedical Sciences Group, UCSD and VA Medical Center, San Diego, CA 92161-6002.

Reinnervation by a foreign motoneuron may strongly influence myosin heavy chain (MHC) expression. MHC content may be controlled by both gene transcription and translation, thus MHC mRNA and protein were examined in the tibialis anterior (TA) and soleus (Sol) following denervation and reinnervation. The sciatic nerve of adult rats was transected and repaired by either epineurial sutures or a silicone tube (10/group). Following 8 or 32 wks of recovery, the muscles were excised, weighed, and frozen. MHC protein content was quantified by SDS-PAGE and mRNA levels with the RNase Protection Assay which allows comparison of relative levels of the 4 adult MHCs within the same sample. Control Sol contains predominately type I MHC, however, 8 wks following nerve injury, there were increases in IIa and IIx MHC mRNAs without corresponding increases in the proteins. However, by 32 wks, increases in both fast MHC mRNA and protein, especially IIx, were found with concomitant decreases in slow MHC. The relative amounts (% of total) of IIx MHC mRNA and protein increased from ~5% in control Sol to ~45% at 32 wks with tube repair. Similarly, 32 wks after tube repair in the TA, IIx MHC mRNA and protein increased primarily at the expense of IIb MHC. In control TA, IIb mRNA content was ~77% while 32 wks following tube repair, IIb mRNA ranged from 4 to 16%. These findings emphasize the role that motoneurons play in regulating MHC expression. The timing of these changes indicates that isoform switching can be detected at the mRNA level considerably before detection of the new protein. This work was supported by the Research Service of the VA.

654.2

MHC PROTEIN AND mRNA EXPRESSION IN RAT SOLEUS MOTOR UNITS AFTER SCIATIC NERVE INJURY. D.J. Pierotti*, and S.C. Bodine-Fowler. Dept. of Orthopaedics, UCSD and VA Medical Center, San Diego, California 92161-6002.

Although the nerve has been shown to affect the myosin heavy chain (MHC) isoform expressed in a muscle, little is known about the expression of the MHC mRNA transcripts. We chose to examine the influence of the nerve by studying MHC protein and mRNA expression in motor units of the rat soleus (Sol). Sciatic nerves of adult female rats were transected followed by epineurial suture repair. After a period of 16 weeks, motor units from the Sol were functionally isolated, physiologically tested, and glycogen depleted. Motor unit fibers were identified in serial cross-sections that had been labeled with MHC antibodies and mRNA probes. Previously, we demonstrated that only 10% of the neurons reinnervating the Sol were from the original motor pool and that the Sol was reinnervated by twice as many motoneurons (Bodine-Fowler et al., Neurosci. Abstr. 20: 256, 1994). Whole muscle tetanic force was reduced from normal, as was the average fiber size. Motor units (n=6: 2 slow, 2 IIa, and 2 IIx) ranged in force production from ~15 to 180 mN, in contrast to the 28 to 100mN range in control motor units. Contraction time ranged from 12 to 30 msec, as opposed to 25 to 88 msec in control motor units. Only 3 of the 6 motor units were fatigue resistant. Type 2x MHC, normally not found in rat Sol, was abundant in all muscles tested. The fibers in each of the motor units were composed of one type of MHC. Interestingly, each of the visualized units expressed only one mRNA transcript, though a large number of non-motor unit fibers contained two or more transcripts. These results indicate that after nerve injury motor unit fibers have acquired a similar degree of homogeneity with respect to protein and mRNA expression. This work was supported by the Research Service of the VA.

654.3

MYOSIN HEAVY CHAIN COMPOSITION OF SOLEUS MUSCLES IN SPINAL CORD INJURED AND CONTROL HUMANS. R.J. Talmadge, S. Harkema, K. Day, D. Lowry, B. Dobkin, and V.R. Edgerton*. Departments of Physiological Science and Neurology, UCLA, Los Angeles, CA 90024.

Several studies have demonstrated in humans that following a spinal cord injury (SCI) the muscles innervated by motoneurons below the level of the lesion become composed almost entirely of type II fibers, as assayed by myofibrillar ATPase histochemistry. It is now known that each of the three fiber types (types I, IIA, and IIB), as defined by the pH sensitivity of the myofibrillar ATPase histochemical stain in normal muscle, corresponds to a specific myosin heavy chain (MHC) isoform (types I, IIa, and IIb, respectively). However, there have been no previous studies documenting the effects of SCI on MHC composition in humans. We have determined the MHC composition of the soleus muscle in 4 spinal cord injured (from 1 to 3 years post injury) and 4 adult control subjects by using a high resolution SDS-PAGE technique that allows for the quantitative determination of types I, IIa, and IIb MHCs. The proportion of the MHC isoforms in the soleus of control subjects was 68% type I, 25% type IIa, and 7% type IIb MHCs. In contrast, the SCI subjects contained 55% type I, 25% type IIa, and 20% type IIb MHCs. The decrease in type I and the increase in type IIb MHCs after SCI were statistically significant ($p < 0.05$). These data are consistent with a shift from the slower (type I) to the faster (type IIb) MHC isoform, but these changes are not as dramatic as those previously reported for the ATPase based fiber type analysis. These data suggest that the myofibrillar ATPase reaction may overestimate the amount of fast myosin in SCI humans. Supported by NIH Grant NS 16333.

654.4

RHABDOMYOSARCOMMA CELLS TRANSFECTED WITH CONNEXIN-43 SHOW AN INCREASE IN MYOGENIC DIFFERENTIATION. A. Proulx[1], Y.C. Zhang[1], Z.X. Lin[2], J.F. Bechberger[1], P.A. Merrifield[1], and C.C.G. Naus[1]*. [1]Department of Anatomy, Univ. of Western Ontario, London, Canada. [2]Beijing Institute for Cancer Research, Beijing, China.

Gap junctional communication (GJC) has been implicated in many cell physiological processes. Although absent in mature skeletal muscle, studies have shown gap junctions to be present during early stages of myogenesis, indicating their possible role in myogenic processes. Previous reports have shown that communication deficient tumorigenic cells transfected with a gap junction protein gene exhibit an increase in cell differentiation and a decrease in cell proliferation. Rhabdomyosarcomma is a highly malignant tumor of embryonic origin. In our present study, human rhabdomyosarcomma cells were transfected with the cDNA encoding the gap junction protein connexin-43 (Cx43), and clones of varying expression were isolated. Dye-coupling attributable to GJC was proportional to the level of connexin expressed. Furthermore, expression of Cx43 also correlated with increased cell fusion and myosin expression. These results lend further evidence to the possible role of gap junctions during developmental processes such as myogenesis and cell differentiation.

This work has been supported by the Muscular Dystrophy Association of Canada.

654.5

EXPRESSION OF BASIC FIBROBLAST GROWTH FACTOR AND ITS RECEPTORS IN THE HUMAN FETAL MUSCLE. L.Balaci, M.Presta, V.Sogos, M.G.Ennas, P. Dell'Era and F.Gremo*. Sch. of Med., Cagliari, Brescia (Italy)

Basic fibroblast growth factor (bFGF) has been shown to stimulate proliferation and to depress differentiation of myogenic cells. Moreover, it has been proposed to stimulate rigeneration of dystrophic muscle. In order to elucidate its role in the development of the human muscle, we investigated its expression as well as the expression of the four types of FGF receptors (FGF- Rs). In brief, muscles have been dissected out from 10 - 18 week old human embryos and tissues processed for Northern and Western blot analyses. Results demontrated a progressive decrease in bFGF and FGF-Rs content in correlation with age. Immunocytochemical staining of human fetal muscles apparently showed an association between FGF-Rs and satellite cells, further suggesting that bFGF might play a key role in the development of human muscle.

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654.6

TISSUE-ASSOCIATED COUNTERPARTS OF α-2-MACROGLOBULIN AND INTER-α-TRYPSIN INHIBITOR IN MOUSE SKELETAL MUSCLE. De Renzis G.*, Businaro R., Nori S., Toesca A., De Santis E., Evangelisti E., Fumagalli L.. Institute of Anatomy, Catholic University, 00168 Rome, Italy.

Proteinase inhibitors normally regulate proteolysis in numerous tissues and cells. However relatively few data are available in literature about protease inhibitors in normal and diseased muscle. The present experiments deal with the immunohistochemical and immunochemical detection of α-2-macroglobulin (α2M) and inter-α-trypsin inhibitor (ITI), two major protease inhibitors in mouse skeletal muscle. Immunoperoxidase histochemistry (ABC method) was done on cryostat sections fixed with a mixture of 4% paraformaldheyde plus 0.5 monomeric glutaraldheyde. In all muscles examined (mm. soleus, plantaris and extensor digitorum longus) α2M-related immunoreactivity occurred widespreadly in extracellular structures (peri-endomysium, blood vessel wall) as well as inside about a half of the muscle fibers. This localization pattern did not change following extensive *in vivo* perfusion (with PBS) of deeply anesthetized mice to remove serum contamination. Western blotting experiments were negative on homogenates of perfused muscles (soluble fraction), but three specific bands (180,165,35 KDa) resulted in detergent-solubilized extracts (0.3% Triton X-100). These results indicate that i) our perfusion procedure was effective in removing serum (soluble) α2M from muscle, and ii) skeletal muscle contains tissue-associated α2M. In contrast to the above results, ITI localization was restricted to the extracellular matrix both in normal and perfused muscle, with two typical bands of 220 and 180 KDa detected by immunoblotting experiments. The present results suggest that in skeletal muscle some proteinase inhibitors may have a tissue-associated counterpart with different localization and physiological roles.

654.7

IMMUNOSTAINING OF ISOLATED ADULT RAT SKELETAL MUSCLE FIBERS WITH ANTI-NA PUMP ANTIBODIES. S. Corey Specht* and M. Santiago-Sanchez. Dept. Pharmacology and Inst. Neurobiology., Univ. PR Sch. Med, San Juan, PR 00936.

The organization of Na pump molecules on the surface of adult skeletal muscle was examined in fibers of the rat *digitorum brevis* muscle. Individual fibers were isolated with dispase and collagenase at 37°C for 2 h, relaxed in 30 mM KCL followed by 2M formamide, fixed in 2% paraformaldehyde, and finally immobilized on poly-l-lysine-coated slides under microscopic observation. Tetramethylrhodamine-α-bungarotoxin was used to identify the motor endplate. Myonuclei were identified with Hoechst 33258. Na pump α1 and α2 subunits were tagged with monoclonal antibodies developed by K. Sweadner (Mass. General Hospital), and goat-anti mouse IgG conjugated with rhodamine. Both isoforms of the Na pump were present diffusely on the surface, but absent from large circular areas directly over the bulging sub-sarcolemmal nuclei. When expression was up-regulated by administration of high doses of insulin, Na pump was also seen in fine lines with occasional clumping. [NIGMS grant SS RR 08224]

654.8

ION-DEPENDENT ALTERATIONS IN METABOLIC GENE EXPRESSION IN SKELETAL MUSCLE: S.A. Vali, P. C. Tennant, R. C. Carlsen, R. C. Tait, F. A. Gorin*. Dept. of Neurology, Univ. of California, Davis, CA 95616.

It is well established that metabolic properties of the skeletal muscle fibers are modulated by neurally imposed patterns of contractile activity. Previously we have demonstrated that either denervation, inhibition of neural impulses or blockade of neuromuscular transmission reduce steady state mRNA transcript levels of muscle glycogen phosphorylase (mgp) to 40-60% of controls. These *in vivo* manipulations are associated with increased sarcolemmal permeability to sodium and resulting depolarization. Recently, we have utilized a spontaneously contracting skeletal muscle cell line as an *in vitro* model to examine the cellular mechanisms that modulate mgp levels with inhibition of neuromuscular activity. Myotubes were depolarized by manipulating sodium permeability or potassium gradients. Depolarization altered mgp steady state transcript levels as observed *in vivo*. Calcium influx has been shown to regulate the mRNA levels of the subunits of AChR in myotube cultures. But in our system, increasing calcium influx with ionomycin did not alter mgp steady state RNA levels in contrast to α AChR. From this data, it is likely that neural activity regulates mgp by different mechanisms than observed for AChR subunit genes in skeletal muscle.

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654.9

EQUATORIAL MORPHOLOGY OF SLOW AND FAST INTRAFUSAL FIBERS IN CHICKEN LEG MUSCLE SPINDLES. Alfred Maier*Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294.

Equatorial regions of chicken leg muscle spindles were examined to review the notion that at midequator their intrafusal fibers present a uniform structure, regardless of fiber type. Based on myosin heavy chain composition, intrafusal fibers were either classified as slow (S and M fibers) or fast (U fibers) (Maier and Zak, Am. J. Anat., 187:338-346. 1990). Staining for α-bungarotoxin was exceedingly rare at the equator; indicating that neither fiber type received a significant motor innervation at the sensory region. Immunostaining for acetylcholinesterase , on the other hand, was present as a continuous or occasionally interrupted layer throughout the equator in either fiber group, but only on the non-synaptic side, away from myosensory junctions. Sensory terminals making contact with fast U fibers were frequently claw-shaped, while those connecting to the slow M and S fibers were typically less curved. The perifibral sheath was shortest in M fibers, and on average, there were fewer myosensory contacts on slow than on fast fibers. In sections which were immunostained for filamentous actin by the phalloidin toxin reaction, at the point where the perifibral sheath was most prominent, actin was restricted to a narrow crescent on the synaptic side. This paucity of actin extended along the equator for a greater distance in slow than in fast fibers, despite a more extensive distribution of slow MHC. As seen in cross sections, slow MHC was concentrated on the periphery of fibers. There are then at the equator of chicken intrafusal muscle fibers some fiber type-specific structural elements, but they are much less pronounced than in mammalian spindles. Physiological experiments are required to determine if these morphological differences relate to unique mechanical properties which can induce distinct and separate components into the afferent discharge.

654.10

MORPHOLOGICAL ADAPTATIONS OF DIAPHRAGM NEUROMUSCULAR JUNCTION TO PROLONGED INACTIVITY. W.Z. Zhan*, Y.S. Prakash, H. Miyata and G.C. Sieck. Depts. of Anesthesiology, and Physiology & Biophysics. Mayo Foundation, Rochester, MN 55905.

Previous models of muscle disuse vary in the extent of nerve versus muscle inactivation, making it difficult to assess inactivity-induced adaptations of the neuromuscular junction (NMJ). We induced inactivation of both phrenic motoneurons and diaphragm muscle in the rat via spinal isolation (SI) at the C2 level. We then combined a three-color fluorescent immunocytochemical technique with 3D confocal microscopy to simultaneously visualize nerve terminals, motor endplates and muscle fiber type in SI diaphragm fibers. Compared to controls, both nerve terminal and endplate planar (2D) and surface (3D) areas were increased on type II fibers, but not on type I fibers. The extent of overlap of pre- and postsynaptic elements of the NMJ increased on type II fibers. Terminal and endplate branch lengths on type II fibers also increased. Endplate gutter depth was unchenged. These results suggest that NMJ inactivation at both pre- and postsynaptic levels leads to a phenotype-dependent expansion of the NMJ on type II fibers, perhaps in an effort to improve neuromuscular transmission. Supported by NIH grants HL34817 and HL37680.

645.11

EFFECT OF AGING ON DIAPHRAGM NEUROMUSCULAR JUNCTION MORPHOLOGY. L.E. Gosselin, Y.S. Prakash, D.B. Masters*, and G.C. Sieck. Depts. of Anesthesiology, and Physiology & Biophysics. Mayo Foundation, Rochester, MN 55905.

Previous studies reported fiber-type differences in morphological adaptations of neuromuscular junctions (NMJs) to aging by comparing limb muscles consisting of predominantly type I or II fibers. Differences in age-related change in activity of these muscles may be a confounding factor in these studies. In the present study, we assessed age-related changes of the NMJ in type I and II fibers of the rat diaphragm muscle which maintains consistent inspiratory-related activation throughout life. In 6- and 24-month old rats, nerve terminals were labelled with an antibody to protein gene product 9.5, and motor endplates were labelled with α-bungarotoxin. Fiber type was identified using an anti-fast myosin antibody. Labelled NMJs were imaged in 3D using confocal microscopy. When corrected for fiber diameter, only type II fiber NMJs showed significant changes with aging. Nerve terminal and endplate planar (2D) and surface (3D) areas expanded, and the number of nerve terminal and endplate branches increased. In older animals, the number of axon collaterals also increased. These morphological adaptations may relate to changes in neuromuscular transmission with aging. Supported by NIH grants HL34817 and HL37680.

645.12

CHARACTERIZATION OF A PROGRESSIVE MUSCLE DEGENERATION MUTATION IN *DROSOPHILA*. B.Clendening*, T. Tubon, J. E. Allen and G. L. Harris. Department of Biology, San Diego State University, San Diego, CA 92182.

We are using an enhancer trap analysis to identify and characterize mutations in *Drosophila* which disrupt the function of the indirect flight muscles (IFMs) of adult flies. One enhancer trap line has been used to generate a single complementation group of recessive mutations on the second chromosome which cause a progressive degeneration of the IFMs. Upon eclosion, flies homozygous for the mutation, have a normal appearance and exhibit normal walking, jumping and flying behavior. Soon after eclosion, the mutant flies become progressively impaired in their flight ability such that by four weeks most mutant flies are unable to sustain normal flight. The ultrastructure of the IFM fibers becomes progressively disrupted over the same time period. Severe fraying of the myofibrillar lattice and extensive vacuolization within the sarcoplasm are observed. Other skeletal muscles of the thorax, such as the jump muscle, appear unaffected by the mutations. There is no apparent loss of any major muscle protein band on a single dimensional gel analysis of the mutant flies. In addition to the muscular degeneration, the lifespan of the mutant flies is decreased by several weeks compared to control flies. We have undertaken a molecular analysis in order to isolate the gene(s) which underlie the mutant phenotype. Overlapping genomic and one class of cDNA clones have been isolated which identify transcripts of 1.1 and 2.1 kb on Northern blots of poly A^+ RNA from wild type flies. (Supported by a fellowship to B.C. and a Grant-In-Aid to G.H. from the American Heart Association, California Affiliate).

645.13

INTERCELLULAR COMMUNICATION MONITORED BY PROPAGATION OF CALCIUM WAVES IN CULTURED COLONIC SMOOTH MUSCLE CELLS. S.H. Young*, H.S. Ennes, and E. Mayer. CURE: VA/UCLA Gastroenteric Biology Center, Neuroenteric Biology Group; Depts. of Physiology and Medicine, UCLA and West Los Angeles VA Med Ctr., Los Angeles, CA 90073.

Single muscle cells respond to a light mechanical stimulus with a local increase in intracellular calcium which can propagate throughout the cell and into several adjacent cells. Smooth muscle cells were isolated from the circular and longitudinal layers of the rabbit distal colon, and placed into culture. Stimulus was delivered by a fire polished glass pipette (2-4 μm diameter) connected to an electronically controlled micropositoner (Eppendorf) used to displace the plasma membrane (2μm depression, 0.5 sec duration). The calcium increase starts local to the stimulation point, but if the response exceeds 100 nM above resting levels, the increase will propagate as a wave throughout the cell (intracellular velocity 13.2 μm/sec). Recovery rates to resting levels are highly variable, showing both single double time constants in the range τ= 7-300 sec. When multiple cells are in contact, the wave continues to propagate into as many as 5 adjacent cells (intracellular velocity 17 μm/sec) without large degradation in amplitude. The propagation is likely the result of diffusion of a "message" substance which releases calcium from intracellular stores, and not calcium itself: The wave can propagate through cells which do not respond, and into adjacent cells which do exhibit an increase in calcium. Propagation occurs in zero calcium external solution (21 μm/sec). After treatment with thapsigargin (2μM), a Ca2+-ATPase inhibitor which should deplete intracellular calcium stores, the stimulated cell responds, but intercellular propagation is blocked. Treatment with U-73122, an inhibitor of phospholipase C dependent processes, including production of IP_3, prevents intracellular propagation, indicating that IP_3 may be the messenger molecule. Supported by NIH Grant DK40919-05

645.14

NITRIC OXIDE SIGNALING AT THE NEUROMUSCULAR JUNCTION. D.S. Chao, J.E. Brenman, G.L. Yount*, and D.S. Bredt. Dept. of Physiology, UCSF, San Francisco, CA 94143.

Nitric oxide (NO) is synthesized in skeletal muscle by neuronal NO synthase (nNOS), which is localized beneath the sacrolemma of fast twitch muscle fibers. We now show by immunohistochemistry that nNOS is enriched at the mammalian neuromuscular junction (NMJ). NOS is tethered to the NMJ by a glycoprotein complex formed around dystrophin, the gene mutated in Duchenne muscular dystrophy, and utrophin, an autosomal homolog of dystrophin localized specifically at NMJs in skeletal muscle. We also find that a downstream effector of NO, cGMP-dependent-protein kinase Iα (cGKIα), is localized exclusively at the NMJ in rat skeletal muscle. Both nNOS and cGKIα staining persists two weeks after denervation demonstrating that both are localized in the muscle and not the nerve terminal. nNOS staining at the NMJ is reduced in *mdx* mice and absent in nNOS knockout mice while cGKIα staining persists in both of these animal models. While nNOS has previously been found only on sarcolemma of fast twitch muscle fibers, we find nNOS staining at the NMJ in both fast and slow twitch fibers. These data suggest roles for NO and cGMP as modulators of transmission at the NMJ.

LIMBIC SYSTEM AND HYPOTHALAMUS: AMYGDALA AND HYPOTHALAMUS

655.1

IONIC MECHANISMS OF METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION IN MAGNOCELLULAR NEURONS OF THE HYPOTHALAMIC SUPRAOPTIC NUCLEUS. L.A. Schrader* and J.G. Tasker. Neuroscience Program and Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118.

Activation of metabotropic glutamate receptors in magnocellular neurons of the hypothalamic supraoptic nucleus (SON) results in a depolarization and a decrease in membrane conductance (Schrader and Tasker, Soc. Neurosci. Abstr. 20: 347, 1994). We have investigated the ionic mechanisms of these effects with whole-cell recordings in coronal slices (400 μm) of rat hypothalamus using the metabotropic glutamate receptor agonist *trans*-(+)-1-amino-1,3-cyclopentane-dicarboxylic acid (*trans*-ACPD) and selective ion channel blockers. Bath application of the Na^+-channel blocker tetrodotoxin (1-3μM) had no effect on the inward current or on the decrease in conductance evoked by *trans*-ACPD. However, when K^+ channels were blocked by replacing K^+ in the patch pipette with an equimolar concentration of Cs^+ and by including tetraethylammonium (10-20 mM) in the bath perfusion the decrease in conductance was attenuated or blocked completely. In some cases, when K^+ channels were blocked, *trans*-ACPD caused an increase in membrane conductance, which was attenuated by adding the Ca^{2+} channel blocker Cd^{2+} (200-250 μM) to the bath. These results indicate that metabotropic-receptor activation causes a decrease in membrane conductance and an inward current in SON magnocellular neurons by closing K^+ channels and by activation of a separate cationic current which has a Ca^{2+} component. This work is supported in part by NIH NS31187.

655.2

RESPONSES OF MEDIAL AMYGDALA (MeA) NEURONS TO ELECTRICAL STIMULATION OF THE SUPRAOPTIC NUCLEUS (SON) AND LATERAL OLFACTORY TRACT (LOT). Q.Z. Yang* and G.I. Hatton. Dept. of Neuroscience, Univ. of California, Riverside, CA 92521

Previous extracellular recording studies have suggested the existence of connections (perhaps reciprocal) between the MeA and the SON including its perinuclear zone (PNZ). Our intracellular recording experiments investigated this potential local circuit involving the MeA, the SON and the PNZ. Also studied was the nature of the input from the olfactory bulbs to MeA neurons, connections for which there is long-standing anatomical evidence. In horizontally cut slices that preserve the ventral amygdalo-hypothalamic pathway, electrical stimulation of the SON/PNZ produced short latency EPSPs or IPSPs in MeA neurons. Five MeA neurons were also antidromically activated by SON/PNZ stimulation, suggesting reciprocal connections between the two regions. Three MeA neurons were synaptically excited by both SON/PNZ and LOT stimulation, indicating convergent inputs. In three MeA neurons, stimulation of the SON elicited EPSPs that were blocked by the oxytocin antagonist, [d(CH_2)$_5$,Tyr(Me)^2,Orn^8]-vasotocin, consistent with recent findings of oxytocin receptors in the MeA. LOT stimulation consistently evoked EPSPs in MeA neurons. These ESPSs were blocked by the non-NMDA receptor antagonist, CNQX (5 μM). In five MeA neurons displaying IPSPs in response to SON/PNZ stimulation, these synaptic responses were abolished by the $GABA_A$ receptor blocker, bicuculline. These results suggest that MeA neurons receive inputs from and project to the SON and its PNZ, and that some of these inputs from the SON are oxytocinergic, probably arising from SON axon collaterals. Also MeA, like SON, neurons receive excitatory input form the olfactory system via the LOT. Supported by NINDS grant NS 16942.

655.3

ACTIVATION OF HISTAMINERGIC INPUTS TO PHASICALLY FIRING SUPRAOPTIC NUCLEUS (SON) NEURONS INCREASES DYE COUPLING: EFFECTS OF H_1-ANTAGONISTS AND cGMP. G.I. Hatton* and Q.Z. Yang Dept. of Neuroscience, Univ. of California, Riverside, CA 92521

Activation of direct olfactory inputs (glutamatergic) to the SON has been shown to increase the incidence of interneuronal coupling in slices from lactating, but not virgin or male rats. Here we studied the influence on coupling of activating another monosynaptic input to the SON, the histaminergic tuberomammillary nucleus (TM) projection, which is maintained intact in our horizontally cut slices. Stimulation of the TM selectively excites phasically firing (vasopressin) cells, an effect which is blocked by H_1-antagonists. In this study, we investigated the effects of TM stimulation and its possible downstream second messenger consequences on Lucifer Yellow (LY) dye coupling among phasically firing cells in male rat SONs. In unstimulated slices, LY injections into 27 cells (1 cell/SON) yielded 18 single and 18 coupled neurons. In slices in which TM was stimulated for 10 min at 10 Hz, 13 injections yielded 4 single and 28 coupled cells, a 185% increase in number of dye filled cells/injection ($p< .02$). LY injections into 19 cells in unstimulated and 21 injections in stimulated slices, both bathed in medium containing 10 μM pyrilamine (H_1-antagonist), yielded similar numbers of coupled neurons. That this coupling effect was blocked by an H_1-antagonist suggested that it might be mediated by guanylate cyclase-cGMP to which H_1 receptors are often linked. In a parallel study, slices were bathed in control medium or medium containing 0.5 mM 8-bromo-cGMP. In control medium, 15 LY injections yielded 10 single and 10 coupled cells whereas, in medium containing cGMP, 19 injections yielded 6 single and 32 coupled neurons, a 142% increase ($p< .02$) over control. Taken together, these results suggest that one mechanism mediating synaptic enhancement of interneuronal coupling involves connexon channel protein phosphorylation via cGMP-related actions. Supported by NINDS grant NS16942.

655.4

CHARACTERIZATIONS OF BURST FIRING PATTERNS IN RAT SUPRAOPTIC NEURONS PERIFUSED WITH CALCIUM-FREE MEDIUM. Z. Li* and G.I. Hatton. *Dept. of Neuroscience, University of California, Riverside, CA 92521.*

The present experiments were to investigate ionic mechanisms underlying burst activities of hypothalamic supraoptic (SON) neurons during perifusion of Ca^{2+}-free medium. Whole-cell recordings were obtained from SON neurons in horizontal brain slices from adult rats. Using electrodes filled with K gluconate solution containing no Ca^{2+} buffers or Ca^{2+}, either phasic or regular firing patterns were seen in SON cells. Following perifusion of a medium containing 0 Ca^{2+} with or without 2 mM EGTA, all 33 SON cells recorded displayed bursting activity. Such bursting was characterized by fast, periodic changes in membrane potential with superimposed spike activity. Membrane potential fluctuations were as much as 20 mV when resting membrane potentials were -70 to -55 mV. Further hyperpolarization (more negative than -70) inhibited burst firing. SON cells displaying phasic firing and depolarizing afterpotentials following spikes in Ca^{2+}-containing medium had more prominent burst activities in Ca^{2+}-free medium. Bath application of TTX (1-2 μM, n=6) or replacement of 83% Na^+ in perifusion medium with Li^+ (n=5) cancelled or reduced membrane potential fluctuations. Abolition of burst by perifusion with Na^+-Ca^{2+} exchanger inhibitors, Ni^{2+} (5 mM, n=7) and Mg^{2+} (10 mM, n=6) was accompanied by a decrease in membrane input resistance. Following intracelluar diffusion of 11 mM BAPTA, bursting patterns were abolished or greatly reduced (n=10). Intracellular or extracellular application of ryanodine receptor blockers, ryanodine (20 μM, 8/8 cells) or ruthenium red (20-40 μM, 4/7 cells) prevented or reduced such activities. These experiments suggest that both enhanced Na^+ influx and Ca^{2+} release from internal stores are involved in formation of bursting patterns in Ca^{2+}-free perifusion medium and provide further evidence for functional roles of releasable Ca^{2+} from internal stores in SON neurons. Supported by NINDS grant NS16942.

655.5

HISTAMINERGIC SUPPRESSION OF K^+ LEAKAGE CONDUCTANCE IN HYPOTHALAMIC SUPRAOPTIC NEURONS: ROLES OF G-PROTEIN AND PROTEIN KINASE. C. Decavel*, Z. Li and G.I. Hatton. *Dept. of Neuroscience, University of California, Riverside, CA 92521.*

Histamine (HA) has been suggested to function as a neuromodulator and/or neurotransmitter in the rat supraoptic nucleus (SON). Our previous studies have shown that HA can directly depolarize SON neurons by activating membrane H_1 receptors and this action involves Ca^{2+}-independent intracellular processes and a reduction in membrane conductance. Here we investigated ionic mechanisms and intracellular signal transduction involved in HA-induced prolonged depolarization.

Whole-cell recordings were obtained from SON neurons in horizontal brain slices from adult male rats. In medium containing 0 mM Ca^{2+}, 2 mM Co^{2+} and 1-2 μM TTX, with internal Ca^{2+} chelation (11 mM BAPTA), bath application of HA induced an apparent inward current in 15/20 cells tested. The peak of inward current evoked by 1-10 μM HA at holding potentials around -50 mV was 27.3±0.3 pA (Mean±SEM). Ramp voltage tests revealed that this inward current had a reversal potential of -90.1±3.8 mV (n=10). Current subtraction resulted in a current which showed little voltage dependence. Increased $[K^+]_i$ or introduction of K^+ channel blockers in the medium attenuated or abolished HA-induced inward current at membrane potentials close to -50 mV. When external Cl^- concentration was reduced, HA-induced inward current was still seen in 5/7 cells tested. Neither inward current nor change in conductance was observed following bath application of HA in 11/12 cells recorded using patch pipettes containing GDP-βs, and in 7/8 cells using pipettes containing GTP-γs. When electrodes containing the protein kinase inhibitor, H_7 (0.5 mM) were used, inward current with a reversal potential close to -90 mV was seen only in 1 of 11 cells tested. These results suggest that HA depolarizes SON neurons, in part at least, by inhibiting a K^+ leakage current mediated by G-protein and protein kinase. Supported by NINDS grant NS16942.

655.6

PHYSIOLOGICAL MAPPING OF LOCAL INHIBITORY NEURONS WHICH PROJECT TO THE PARAVENTRICULAR NUCLEUS IN RAT HYPOTHALAMIC SLICES. **C. Boudaba, K. Szabo and J.G. Tasker*. Dept. of Cell & Molecular Biology, Tulane University, New Orleans, LA 70118.**

Local circuit interactions may play an important role in the generation of the rhythmic bursting patterns of hypothalamic neurosecretory neurons. Neurons of the paraventricular nucleus (PVN) receive local synaptic inputs from GABA neurons outside the PVN (Tasker and Dudek, J. Physiol. 469: 179, 1993). The aim of the present study was to determine the origin of the local inhibitory inputs to PVN magnocellular and parvocellular neurons. Postsynaptic potentials were recorded in PVN cells in coronal, horizontal and parasagittal hypothalamic slices (400-500 μm) with sharp electrodes; local neurons were selectively stimulated by focal drop application of glutamate (10-50 mM). Cells were classified as magnocellular or parvocellular neurons during on the basis of electrophysiological criteria (Tasker and Dudek, J. Physiol. 434: 271, 1991), and were injected with biocytin (1%) for subsequent immuno-identification with a neurophysin antibody (generously provided by Dr. A.G. Robinson). As in the previous study, we found that both magnocellular and parvocellular neurons of the PVN received local synaptic inputs from cells outside the PVN. The synaptic responses were caused by GABA release since they were blocked by bicuculline (10-50 μM). The main sources of local inhibitory inputs to the PVN were the region surrounding the fornix, including the bed nucleus of the stria terminalis, and the dorsomedial hypothalamus. Other regions which provided weaker inhibitory inputs to PVN cells were located in the anterior hypothalamus. These local GABA neurons may serve as an inhibitory relay to neurosecretory cells from limbic structures, since the limbic system has an inhibitory influence on neurosecretion and afferent projections from limbic structures terminate outside the PVN. This study was supported by NIH-NS31187, NSF IBN-9315308, and a grant from the LA Board of Regents.

655.7

SYNAPTIC INTERACTIONS BETWEEN AMYGDALO-STRIATAL AFFERENTS AND PARVALBUMIN AND CALRETININ INTERNEURONS WITHIN THE RAT VENTRAL STRIATUM. T.Rudkin[1], A.Sadikot[1], Y.Smith[2] and A.J.Morin[1]*, *1.Dept.Neurology/Neurosurgery, MNI, McGill University, Montreal, PC; 2. Laboratoire de Neurobiologie, Hôpital Enfant-Jésus, Laval University, Quebec City, PC.*

Afferent input from the basolateral amygdala (BLA) to the ventral striatum is thought to influence voluntary responses to novel and reinforcing stimuli *(Everitt and Robbins, 1992)*. Evidence has suggested that dopamine can modulate this response and that this is effected via synaptic convergence of nigral and amygdalar input upon striatal projection neurons *(Johnson, et al, 1994)*. Interneuron populations within the striatum may represent another substrate for modulation of amygdalar input. Two distinct populations of interneurons, identified by their immunoreactivity to the calcium binding proteins parvalbumin (Pv) and calretinin (Cr), have been localized within the rat ventral striatum. Both of these populations have been shown to receive asymmetric synapses *(Lapper, et al, 1992* and *Bennet and Bolam, 1993)* and are GABAergic *(Kubota, et al, 1993)* This study examines whether Pv and Cr interneurons are targets of the amygdalo-striatal projection.

Injections of the anterograde tracer biotinylated dextranamine were made into the BLA. Areas of overlap between the amygdalo-striatal projection and Pv and Cr positive cells were analyzed by electron microscopy. Preliminary results indicate the presence of asymmetric synapses between amygdala terminals and the dendrites of Pv neurons. Synaptic input to Cr neurons has yet to be identified. Positive results from this study will support the hypothesis that these neurons mediate feed-forward inhibition of the excitatory signals arising from the amygdala.

655.8

LOCALIZATION OF VASOPRESSIN-IMMUNOREACTIVITY AND VASOPRESSIN mRNA LABELING IN THE EXTENDED AMYGDALA. G.J. De Vries*, M.A. Miller, W.C.J. Chung. Program in Neuroscience and Behavior, Univ. of Massachusetts, Amherst, MA 01003-7710 and Dept. of Psychiatry and Behav. Sciences, Univ. of Washington, Seattle, WA 98195.

The bed nucleus of the stria terminalis (BST) and the centromedial amygdala show striking similarities in cytoarchitecture, neurochemistry, and neuronal connections and have, therefore, been proposed to belong to one anatomical unit, the extended amygdala (*Alheid et al., 1995*). The vasopressin (AVP) cells of the BST and amygdala illustrate these similarities. They are similar in size and morphology; immunoreactive for estrogen and androgen receptors and for galanin. In addition, AVP expression in both areas depends entirely on gonadal hormones. Here we test whether AVP-immunoreactive (AVP-ir) and AVP mRNA expressing neurons are indeed found in homologous divisions of the BST and amygdala according to the percolation of the extended amygdala proposed by Alheid et al., (1995). In the BST, the majority of the AVP cells were found in the anterior medial BST (BSTMA), the lateral part of the posterior medial BST (BSTMPl), and the intermediate part of the lateral BST (BSTLI). In the amygdala, AVP cells were found in the intraamygdaloid bed nucleus of the stria terminalis (BSTIA), the anterodorsal part of the medial amygdaloid nucleus (MeAD), and the medial part of the central nucleus (CeM). These areas are indeed each others counterparts. The distribution of AVP cells over neurochemically distinct areas within the extended amygdala suggests that these cells may have been exposed to different microenvironments during development. This may explain differences between those cells, such as the AVP neurons in the medial BST being more abundant in males than in females, whereas AVP cells in the lateral BST do not differ in number. *Funded by NSF grant, IBN 9421658 to GJD, and NIA grant AG-10917 to MAM.*

655.9

LOCALIZATION OF BENZODIAZEPINE/GABA$_A$ RECEPTORS IN THE BASOLATERAL AMYGDALA OF THE RAT AND MONKEY: AN IMMUNOHISTOCHEMICAL STUDY. A.J. McDonald* and F. Mascagni. Dept. of Cell Biology and Neuroscience, Univ. of South Carolina School of Medicine, Columbia, SC 29208.

The basolateral amygdala (ABL) has a strong intrinsic inhibitory system mediated by GABA$_A$ receptors and is the main site of the anxiolytic actions of benzodiazepines. In an effort to identify the anatomical substrates for these transmitter and drug actions, immunohistochemical techniques were used to analyse the neuronal localization of the GABA$_A$/benzodiazepine receptor complex (GABAR/BZR) in the rat and monkey ABL.

The overall pattern of GABAR/BZR immunoreactivity was very similar in both species. The neuropil of the lateral nucleus exhibited the most robust staining. GABAR/BZR immunoreactivity was also seen in neuronal perikarya and dendrites where it was localized to the cytoplasm and/or surface membrane. The cell type with the strongest GABAR/BZR immunoreactivity was a subpopulation of small nonpyramidal neurons that had numerous thin dendrites. Other larger nonpyramidal neurons were also stained. Pyramidal neurons in the rat and monkey ABL exhibited light to moderate perikaryal staining that varied in different nuclei.

The results of this study indicate that the pattern of GABAR/BZR immunoreactivity in the neuropil of the rat and monkey ABL closely resemble the distribution of benzodiazepine receptors localized in previous radioligand autoradiographic studies. The finding of intense immunoreactivity in subpopulations of nonpyramidal neurons suggests the existence of disinhibitory mechanisms which may be important for the activation of ABL projection neurons in response to behaviorally significant stimuli. (Supported by NIH Grant NS19733).

655.10

ARE THE PROJECTIONS FROM THE CENTRAL NUCLEUS OF THE MACAQUE MONKEY AMYGDALA TO THE BRAINSTEM GABAERGIC? M.H. Mesches*, A.L. Jongen-Rêlo & D.G. Amaral Ctr Behav Neurosci, SUNY Stony Brook, Stony Brook NY 11794-2575.

The central nucleus of the monkey amygdala (Ce) gives rise to prominent subcortical connections that innervate sensory, endocrine, autonomic, and visceral regions of the brainstem such as the parabrachial nuclei, the dorsal motor nucleus of the vagus and the nucleus of the solitary tract (NTS). While there have been several attempts to determine the chemical anatomy of these projections, relatively little is known concerning their transmitter(s). The present study was prompted by the recent finding in our laboratory that many neurons of the Ce express mRNA for GAD65 and GAD67 and may be GABAergic. Supporting this, Pickel and colleagues (Soc. Neurosci. Abs, 1993) have demonstrated that Ce efferents to the NTS form symmetrical synapses. The present study examined whether cells in Ce, labeled following retrograde tracer injections into the brainstem, were also positive for expression of GAD65 or GAD67. The retrograde markers Fast blue (FB) or WGA-apoHRP-gold (WAHG) were injected into the parabrachial region in Macaca fascicularis monkeys. Tissue was processed by in situ hybridization with probes for GAD65 or GAD67 and with silver intensification of the WAHG. Based on the distributions of FB and GAD positive cells, it appears that some Ce cells which project to the brainstem may be GABAergic. We are currently attempting to confirm this finding quantitatively using double labeled WAHG/in situ preparations. Given that a substantial proportion of the subcortically projecting Ce neurons do express GAD mRNA, it raises the possibility that the amygdala may modulate visceral and autonomic function through an inhibitory GABAergic mechanism.

655.11

AMYGDALOID MODULATION OF MESOPONTINE PERIBRACHIAL (PB) NEURONAL ACTIVITY: IMPLICATIONS FOR AROUSAL A.J.Silvestri and B.S.Kapp*. Dept.Psychol.,Univ. of Vermont,Burlington,VT 05405.

Amygdaloid electrical stimulation in the rabbit produces short-latency activation of auditory responsive neurons located within the mesopontine peribrachial (PB) region (Pascoe and Kapp, 1993). Given the importance of the amygdala and PB region in arousal (Kapp et al.,1992; Steriade et al., 1990), the present study was conducted to determine (1) if the activity of PB auditory responsive neurons in the awake rabbit correlates with the state of arousal as reflected in the cortical EEG and (2) if such neurons are influenced by amygdaloid stimulation. To date, we have been investigating two types of extracellularly recorded auditory responsive neurons. The first, with a spontaneous firing rate of <0.5Hz, responded with a phasic burst of 1-4 spikes to the onset of auditory stimuli. The response of this type of neuron is similar to that of a PGO-on burst neuron (Steriade et al. 1990) which may contribute to thalamic arousal in response to novel stimuli. Neither the stimulus-evoked nor spontaneous activity of these neurons showed any obvious relationship with the state of EEG arousal. Consistent with our previous findings, amygdaloid stimulation produced a short latency (4-13 msec) activation of these neurons. The second type, with a spontaneous firing rate of 2.0Hz, responded with a train of spikes which outlasted the duration of auditory stimuli and continued to respond at an enhanced rate throughout the stimulus-induced EEG desynchronization period. In the absence of stimulus presentations, the spontaneous firing rate of these neurons was greater during periods of EEG desynchronization than during periods of EEG synchronization. Current experiments are investigating whether amygdaloid stimulation influences this type of neuron. The results suggest an amygdaloid influence on PB neurons, some of which may contribute to thalamic arousal. (Supported by NSF 9319699).

655.12

PROJECTION CELLS AND INTERNEURONS OF THE BASOLATERAL (BL) AMYGDALOID COMPLEX HAVE DISTINCT DISCHARGE PATTERNS IN CONSCIOUS BEHAVING CATS J. Dong*, H. Gaudreau and D. Paré Dept. Physiol., Fac. Medicine, Univ. Laval QUÉ. CANADA

Recently (Paré at al., J. Neurophysiol, in press), we correlated the physiological and morphological properties of BL neurons and identified two types of spiny projection neurons (by antidromic invasion). The first cell type prevailed in the BL nucleus and generated spike bursts upon depolarization. The second cell type prevailed in the lateral nucleus and generated slow membrane potential oscillations when steadily depolarized. In contrast to projection cells, aspiny interneurons had a higher spontaneous discharge rate (DR) and generated non-accommodating spike trains during depolarizing current pulses. Here, we verified if these physiological properties translate into discharge patterns that can be recognized extracellularly in behaving cats.

The majority of the cells encountered in the BL nucleus had a low DR but were most active during slow-wave sleep (SWS). These cells generated stereotyped spike doublets or bursts and a high proportion of them were formally identified as projection cells by antidromic invasion from the parahippocampal cortices. A second cell type discharged tonically in waking (W) and REM sleep (10-25 Hz) but displayed phasic DR accelerations (up to 385 Hz) in SWS. Because none of them could be antidromically invaded from the parahippocampal cortices, they are presumed to be interneurons.

In the lateral nucleus, two cell types were recognized. The first behaved like the presumed interneurons of the BL nucleus. In spontaneous conditions, neurons of the second type were silent in all states (W, SWS and REM sleep) but generated antidromic spikes in response to cortical shocks. These findings suggest that in previous extracellular studies of the lateral nucleus, most spontaneously active cells were interneurons. Thus, these data must be re-interpreted. Supported by MRC grant MT-11562.

655.13

RHYTHMIC ELECTRICAL ACTIVITY IN THE AMYGDALA: OBSERVATIONS *IN VITRO* AND *IN VIVO*. H.-C. Pape*[1] and D. Paré[2]. [1]Inst. Physiologie, Otto-von-Guericke Universität, Magdeburg, Germany; [2]Dépt. Physiologie, Université Laval, Québec, Canada.

Despite recent advantages, the operations carried out by the basolateral complex of the amygdala (BLA) remain poorly understood, partly due to the rarity of data on the intrinsic properties of the neuronal constituents. In an attempt to correlate morphological cell types and membrane characteristics, we have combined electrophysiological and staining methods in the BLA of guinea pig and cat *in vivo* and in a slice preparation *in vitro*. The major type of projection neuron in the lateral nucleus, as identified upon antidromic invasion from basal forebrain or ento-perirhinal cortices, possessed a heterogeneous, modified pyramid or stellate morphology with spiny dendrites. The unifying characteristic of these neurons was the propensity to generate slow oscillatory activity, evident as rhythmic deflections of the membrane potential at 2-10 Hz that sculptured spike patterns. Two types of oscillations were distinguished. Both were of an intrinsic, non-synaptic origin, but differed in voltage-dependence, ionic mechanisms and site of generation. A *low-threshold oscillation* occured in a range subthreshold to spike generation, was dependent on a Na-current, and presumably originated at or close to the soma. A *high-threshold oscillation* at the same predominant frequency occured in the suprathreshold range, was primarily mediated through a Ca-conductance, and presumably of a dendritic origin.

Two sets of membrane conductances thus seem to cooperate to produce resonant oscillatory activity at 2-10 Hz in projection neurons of the lateral amygdala, thereby supporting the synchronization of synaptic inputs and action potential output into coherent network oscillations.

655.14

SLOW INHIBITORY SYNAPTIC RESPONSES IN AMYGDALOID NUCLEI. L. Danober* and H.-C. Pape. Institut für Physiologie, Otto-von-Guericke Universität, D-39120 Magdeburg, Germany.

Besides GABA, glycine is the main inhibitory transmitter in the nervous system. In order to investigate the contribution of glycine to synaptic transmission in the amygdala, we combined immunohistochemical and intracellular recording techniques in slices of the guinea pig amygdala *in vitro*. A dense to moderate immunoreactive staining for gephyrin and the α- and ß-subunits of the glycine receptor was observed throughout the amygdala. Local application of glycine to neurons in lateral (LA) and, as previously described[1], central (CE) amygdaloid nuclei evoked a membrane hyperpolarization from rest that reversed at -80 mV. The glycine response persisted during blocked synaptic transmission, was antagonized by strychnine, and injection of K-nitrate positively shifted the reversal potential. Local high-frequent electrical stimulation evoked a slow IPSP in CE neurons, which reversed at -80 mV and was blocked by strychnine[1]. In LA neurons, repetitive stimulation of the external capsule evoked a slow IPSP with similar reversal potential, but of a significantly longer duration and insensitivity to strychnine. This IPSP was substantially increased during blockade of GABA$_A$ and GABA$_B$ receptors, thereby effectively inhibiting burst discharges generally observed during blocked GABAergic transmission.

The results provide evidence for the existence of strychnine-sensitive glycinergic transmission throughout the amygdala and of an additional slow IPSP in the LA nucleus with as yet undefined pharmacological characteristics. Both types of inhibitory synaptic processes may contribute to prevent epileptiform activity during reduced GABAergic transmission. 1, Nose et al., *J. Neurophysiol.* 65, 1227-1241 (1991)

655.15

MICROINJECTION OF TRH ANALOGUE INTO THE CENTRAL NUCLEUS OF THE AMYGDALA STIMULATES GASTRIC CONTRACTILITY IN RATS. N.S. Morrow,*[1] D. Hodgson[2] and T. Garrick.[1,2] CURE/UCLA Gastroenteric Biology Center, Dept. of Veteran Affairs Medical Center,[1] Los Angeles CA 90073, Dept. of Psychiatry and Biobehavioral Sciences,[2] UCLA, Los Angeles, CA 90024.

Changes in gastric contractility following microinjection of thyrotropin-releasing hormone (TRH) analog, RX 77368, into the central nucleus of the amygdala were examined in fasted, urethane-anesthetized rats. Gastric contractility was measured with extraluminal force transducers and analyzed by a computer. Bilateral microinjection of RX 77368 (0.5 μg, 1.0 μg, n= 6 each) dose-dependently stimulated gastric contractility for 10-120 min post-injection when compared to rats microinjected with 0.1 μg RX 77368, 0.1% BSA or RX 77368 into sites adjacent to the central nucleus, p's <.05. Peak responses (0.5 μg, 1.0 μg) occurred 30-40 min post-injection and represented a 15- to 30-fold increase over basal values. In the remaining groups, the gastric contractility force index was not significantly altered from pre-injection values. These results suggests that the central amygdala participates in modulating gastric function during stress-induced challenges. *Supported by a Pilot and Feasibility Award from NIH Center Grant DK 41301 (Animal Core), by VA Merit Review funds and by the UCLA Pyschoneuroimmunology Fellowship Program.

655.16

DISSOCIABLE EFFECTS OF SELECTIVE EXCITOTOXIC LESIONS OF THE AMYGDALA ON INSTRUMENTAL AND PAVLOVIAN COMPONENTS OF A NOVEL CONDITIONED PUNISHMENT PROCEDURE. A.S.Killcross, B.J.Everitt and T.W.Robbins. Department of Experimental Psychology, University of Cambridge, Cambridge, England, CB2 3EB.

SPON: European Neuroscience Association.

It has been widely reported that lesions of either the basolateral (blA) or central (cnA) nuclei of the amygdala will abolish Pavlovian aversive conditioning in rats. A novel conditioned punishment procedure is reported in which rats maintained baseline lever pressing on two levers for pellets whilst superimposed on this food schedule was a second schedule delivering on one lever a response-contingent 10-sec auditory conditioned stimulus (CS) that was terminated by a 0.5-sec, 0.3 mA footshock, and on the other lever a matched neutral auditory CS. Control animals learnt over several sessions to avoid the lever producing the response-contingent aversive CS (CS+ lever), but more rapidly came to show a selective conditioned suppression of lever pressing on both the CS+ lever and the alternative lever only during occurrences of the aversive CS. Responding during the neutral CS was always maintained at the same rate as responding during ITI periods. These two behaviours are due to conditioned punishment of instrumental responding and acquisition of a Pavlovian CS-shock association, respectively. A similar pattern of effects was produced in extinction, demonstrating the importance of the CS-shock association in both of these responses. Quinolinate lesions of the blA produced a slight retardation in the acquisition of the Pavlovian conditioned suppression, but a persistent deficit in the acquisition of instrumental conditioned punishment. Ibotenate lesions of the cnA produced the reverse pattern of effects - a persistent deficit in conditioned suppression, but only a slight retardation in the acquisition of conditioned punishment. The implications of this finding for the role of the central and basolateral nuclei of the amygdala in aversive conditioning are discussed.

655.17

INDUCTION OF RUNNING ACTIVITY AND METABOLIC CHANGE BY THE VENTROMEDIAL HYPOTHALAMUS IN THE RAT. K. Narita[1], M. Nishihara[2], Y. Mori[3]* and M. Takahashi[2]. [1] Dept. of Physiology, Fukui Medical School, Fukui 910-11, [2] Dept. of Vet. Physiology and [3] Dept. of Vet. Ethology, Univ. of Tokyo, Tokyo 113, JAPAN.

We have revealed that injection of kainic acid (KA) into the ventromedial nucleus of the hypothalamus (VMH) exclusively elicited running activity. In this study, first we investigated the involvement of KA type glutamate receptors in the VMH in the metabolic changes during running activity originating in the VMH. Next, efferent pathways of these KA-sensitive neurons in the VMH were examined by means of multiunit activity (MUA) recording technique.

Injection of KA into the VMH of conscious rats resulted in an increase in plasma glucose, norepinephrine (NE) and epinephrine (E), as well as running activity. Then to prevent the occurrence of exercise, rat was anesthetized by urethane and KA was injected into the VMH. In this condition, KA also increased plasma glucose, NE and E. A transient increase in MUA of subthalamic (SLR) and mesencephalic (MLR) locomotor regions were also observed.

From these results we conclude that KA type glutamate receptors in the VMH are involved in inducing running activity and, simultaneously, in activating the sympathetic nervous system with a resultant increase in blood glucose to supply energy substrate during running activity. It was also suggested that both SLR and MLR are involved in running activity originating in the VMH.

655.18

CHANGES IN LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) FOLLOWING INJECTIONS OF A $GABA_A$ ANTAGONIST INTO THE DORSOMEDIAL HYPOTHALAMUS OF RATS. T.J. Sajdyk*, A.Shekhar, J.S. Katner, W.J. McBride and E. Chernet Dept. of Psychiatry, Indiana Univ. Med. Center, Indianapolis, Indiana 46202.

Previous studies have shown that rats injected with a $GABA_A$ antagonist into the dorsomedial hypothalamus (DMH) exhibit panic-like responses (Shekhar, Biol. Psychiatry, 36:748-58;1994). The purpose of this study was to identify other neuroanatomical sites possibly involved in mediating this panic-like response using the [^{14}C] 2-deoxyglucose (2-DG) autoradiographic method (Sokoloff et al., J. Neurochem.,28:897-916;1977). Rats were fitted with femoral arterial and venous catheters as well as unilateral, chronic injection cannulae into the DMH. They were injected with either 20 pmol/100 nl of BMI or artificial cerebrospinal fluid (a-CSF, 100 nl) into the DMH along with intravenous injection of 2-DG (125 uCi/Kg). The ensuing physiological response was recorded and series of blood samples were taken to determine plasma glucose and [^{14}C] concentrations. Rats were sacrificed, their brains serially sectioned and regional LCGU values were determined. A significant increase in LCGU on the BMI injected side were seen in the DMH; lateral, paraventricular, arcuate and posterior hypothalamic nuclei; CA1 region; and central gray. A significant decrease in LCGU was observed in the basolateral amygdala. These results indicate that activity in several discrete nuclei are altered during the panic-like response induced by BMI injections into the DMH of rats (Supported by MH 45362).

655.19

NOREPINEPHRINE RELEASE FOLLOWING $GABA_A$ RECEPTOR BLOCKADE IN THE DORSOMEDIAL HYPOTHALAMUS OF CONSCIOUS RATS: AN IN-VIVO MICRODIALYSIS STUDY. J.S.Katner* A.Shekhar, Sajdyk, T.J., R.R.Kohl, Dept. of Psychiatry, Indiana University Medical Center, Indianapolis, Indiana 46202.

Blockade of $GABA_A$ receptors by locally injecting the antagonist bicuculline methiodide (BMI) into the dorsomedial hypothalamus (DMH) causes a panic-like response which includes increases in heart rate (HR), blood pressure (BP), respiratory rate, as well as "anxiety". Rats experiencing "anxiety" in a fear-potentiated startle test have shown increases in norepinephrine (NE) tissue levels in the DMH. Using in-vivo microdialysis, the present study sought to determine changes in extracellular NE levels following superfusion of different concentrations of BMI into the DMH. Rats were implanted with femoral arterial catheters and microdialysis probes into the DMH. After 24 hours, the DMH of conscious rats were perfused with either 100, 150 or 200 mM BMI solutions via the microdialysis probe. HR and BP responses were recorded and the extracellular levels of NE in the DMH were determined from the perfusates by using high pressure liquid chromatography. Rats receiving BMI injections showed dose-dependent increases in extracellular NE levels in the DMH. These results suggest that in the DMH, increased NE release may be closely connected with $GABA_A$ receptor blockade and the associated panic-like response. (Supported by MH 45362)

656.1

SELECTIVE DISTRIBUTION OF LACTATE DEHYDROGENASE ISOENZYMES IN NEURONS AND ASTROCYTES: EVIDENCE FOR ACTIVITY-DEPENDENT METABOLIC TRAFFICKING

P. Bittar[1], Y. Charnay[1], M. Kiraly[2]*, L. Pellerin[2], C. Bouras[1] and P.J. Magistretti[2]. [1]Département de Psychiatrie, Université de Genève and [2]Institut de Physiologie, Université de Lausanne, Switzerland.

We have observed that glutamate stimulates in a concentration-dependent manner (EC_{50} = 78 μM) glucose uptake into and lactate efflux from mouse cortical astrocytes by a mechanism involving a Na^+-dependent glutamate transporter [LP & PJM (1994) *PNAS* **91**:10625-10629]. Lactate was shown to originate from extracellular glucose. Glutamate uptake did not affect glucose uptake or lactate release in neurons however. Since glutamate is the main excitatory neurotransmitter released by activated neurons, we postulated that lactate produced by astrocytes could serve as an energy fuel for neurons during period of activation. Production and utilization of lactate require the presence of the enzyme lactate dehydrogenase (LDH). Five major LDH isoenzymes have been described. At one end, the LDH-1 (heart type) isoenzyme favors the formation of pyruvate from lactate, while the LDH-5 (muscle type) isoenzyme preferentially catalyzes the reaction in the opposite direction. We have developed and characterized two antisera (Abs) raised against the LDH-1 and -5 subunits from rabbit. The specificity of each antiserum was verified by immunohistochemistry , immunoblot and western blot analysis. Immunohistochemistry on 10 post-mortem control human cases revealed a differential cellular distribution in the hippocampus: neurons were exclusively stained with the anti-LDH-1 Ab while astrocytes were stained by both Abs. These observations are consistent with the idea that neurons process lactate to pyruvate which can then enter the TCA cycle, while astrocytes can glycolytically produce lactate from glucose; the data also support the view of a metabolic exchange between astrocytes and neurons *in vivo* especially during activation.

656.2

THE DIRECT EFFECTS OF ALTERED GLUCOSE CONCENTRATION ON THE ISOLATED RAT MIDDLE CEREBRAL ARTERY (MCA). M.W.G. Swafford, M.Y. Eichler, R.M. Bryan, Jr.* Dept. of Anesthesiology, Baylor College of Medicine, Houston, TX 77030

The direct effects of altered glucose concentration was studied in cerebral arteries. Rat MCAs were isolated, immersed in a bath (37° C) containing physiological saline solution, pressurized to 85 mm Hg, and luminally perfused. Each MCA was magnified with an inverted microscope and the image displayed on a video monitor. Changes in the diameter were measured after altering the luminal and abluminal glucose concentrations.

Decreasing the glucose from 5.5 mM to 1.5, 1.0, and 0.5 mM for 1.5 hours each had no effect on the diameter of the arteries. When all the glucose was removed for 1.5 hours, the MCAs dilated by 23% [252 ± 9 μm to 311 ± 3 μm ($p<0.05$, n=7)]. When all Ca^{+2} was removed from the bath, the MCAs further dilated to 35 ± 5 % of control indicating that maximum dilation did not occur as a result of hypoglycemia alone. When glucose was restored (100 mg/dl), the MCA diameters returned to control. Removal of the endothelium did not alter the response of the isolated MCAs to reductions in glucose. Furthermore, inhibition of the ATP sensitive K^+ channels with 10^{-4} M glibenclimide did not alter the response to glucose.

We conclude that isolated MCAs were relatively resistant to hypoglycemia since all glucose had to be removed before dilations occurred. This is in contrast to cerebral blood flow in intact rats which shows a dramatic increase when plasma glucose falls below 2.5 mM. The direct effects of hypoglycemia on the MCA did not involve endothelial vasodilatory mechanisms or ATP sensitive K^+ channels. (PHS PO1 NS 27616).

656.3

HYPERGLYCEMIA DOES NOT ALTER REGIONAL CEREBRAL BLOOD FLOW (rCBF) OR REGIONAL CEREBRAL BLOOD OXYGENATION (rCBO) CHANGES IN CORTICAL SPREADING DEPRESSION (CSD) IN RATS. U.Lindauer*, T.Wolf, A.Villringer, U.Dirnagl. Dept. of Neurology, Humboldt University Berlin, Germany.

Cortical spreading depression may play a role in the pathology of migraine and cerebral ischemia. The event of CSD, as an intense stimulus of energy metabolism, is in the normal brain followed by marked increases in rCBF, rCBO and tissue lactate concentration. Glucose is the main substrate of energy production in the brain. We tested the hypothesis as to whether glucose may be involved in the mediation of CSD related changes in rCBF and rCBO under normoglycemic and hyperglycemic conditions. In parietal cortex of α-chloralose anesthetized, ventilated rats under full physiological control, we measured rCBF with laser Doppler flowmetry (LDF) through the thinned bone. rCBO (oxyhemoglobin: HbO_2, deoxyhemoglobin: Hb) and oxidized mitochondrial cytochrome aa_3 (CytO) were measured through the intact skull with near infrared spectroscopy (NIRS). The DC potential was monitored epidurally. CSD was induced every 15min by topical application of KCl 10mm anterior from the measuring site. After 1h of normoglycemic CSD recording, hyperglycemia was induced by i.v. glucose application (1ml of 20% glucose solution as a bolus, followed by infusion of 2ml/h 40% glucose solution). A drop in blood pH due to hyperglycemia was balanced by mild hyperventilation. During hyperglycemia CSD was induced every 15min for 1h. Results are summarized in the table:

n=4, *p<0,05 mean ± SD	blood glucose (mmol/l)	bas. rCBF (%)	Δ rCBF (%)	Δ HbO_2 (arb.units)	Δ Hb (arb.units)	Δ CytO (arb.units)
normoglycemia	5.1 ± 0.7	100	+524 ± 98	+35 ± 3	-11 ± 5	-6.2 ± 0.2
hyperglycemia	18.4 ± 3.3*	91 ± 12	+540 ± 95	+35 ± 6	-10 ± 4	-6.6 ± 1.6

rCBF and rCBO responses to CSD were not affected by hyperglycemia, which points against a role of glucose in the coupling of metabolism and rCBF during CSD. (Supported by the Deutsche Forschungsgemeinschaft).

656.4

RELATIVE CONTRIBUTIONS OF GLYCOLYSIS AND OXIDATIVE PHOSPHORYLATION TO METABOLISM OF PRIMARY HIPPOCAMPAL CULTURES, AS ASSESSED BY MICROPHYSIOMETRY. J.A. Trafton, O.A. Ajilore and R.M. Sapolsky.* Biology Dept. Stanford University, Stanford, CA 94305.

Microphysiometry is a technique which measures real time changes in proton efflux rate in cultured cells or tissue slices. It is generally interpreted as measuring metabolic rate, based on the idea that metabolic processes produce hydrogen ions as lactic and carbonic acid in proportion to activity. A problem in interpretation occurs, in that glycolysis contributes 6 times as many protons/ATP as does oxidative phosphorylation. Thus, shifts in the balance between oxidative and glycolytic metabolism would change acid production, which might be wrongly interpreted as an altered metabolic rate. To distinguish between the glycolytic and oxidative contributions, we compared proton efflux rates measured in cultures given glucose and pyruvate as substrate versus pyruvate alone. At pH 7.4, approximately 90% of total metabolism was attributable to glycolysis with only 10% due to oxidative phosphorylation. Acidosis has been shown to inhibit NMDA receptor currents and to protect cultured neurons from excitotoxic insults; commensurate with that, we found that acidosis decreased proton efflux rates in cultures. We explored whether this decrease represented a true metabolic decline. While significant declines in proton efflux rate at lower pH's were still obvious in cultures restricted to oxidative metabolism, a shift toward oxidative metabolism was seen following acidification. At pH 6.4, glycolysis contributed less than 60% of total metabolism. Acidity still decreased the rate of proton production under conditions bypassing glycolysis, with a decline to < 50% of pH 7.4 levels at pH 6.4. This suggests that while acidity indeed decreases metabolic rate, it also shifts energy production towards oxidative metabolism. While the implications of this are unclear, it hints at the complexity of acid's metabolic effects and highlights the need for caution in interpretating microphysiometric data.

656.5

AUTOREGULATION OF CHOROID PLEXUS BLOOD FLOW DURING ACUTE HYPERTENSION. C. A. Hathaway, R. Du, S. B. Waller*, J. L. Williams. Univ. of South Dakota School of Medicine, Vermillion, SD 57069

Autoregulation of cerebral blood flow (CBF) has been well characterized. In contrast, autoregulation of choroid plexus (CP) blood flow (BF) has not been examined. The goal of this study was to determine whether BF to CP autoregulates during moderate increases in mean arterial pressure (MAP).

BF was measured with radioactive microspheres in anesthetized rabbits. In 5 animals, MAP to the head was increased 39 ± 10 mmHg (mean ± SD) by aortic occlusion. BF to CP of the lateral ventricle was unchanged by occlusion (347 ± 148 to 388 ± 85 ml/min/100g). In contrast, occlusion increased BF to the fourth ventricle (216 ± 37 to 428 ± 163 ml/min/100g, p=0.054). CBF was unchanged (78 ± 35 to 95 ± 34 ml/min/100g).

In other rabbits, MAP was increased 36 ± 8 mmHg by IV norepinephrine. BF to CP of the lateral ventricles was similar during control and norepinephrine (288 ± 173 to 259 ± 98 ml/min/100g). BF to CP of the fourth ventricle also was not changed (276 ± 112 to 307 ± 256 ml/min/110g) by norepinephrine.

In 3 rabbits, we measured the rate of autoregulation of BF of CP of lateral ventricle with laser Doppler flowmetry during aortic occlusion, which increased MAP 59 ± 17 mmHg. During aortic occlusion, CPBF and CBF increased 20 ± 7% and 45 ± 8% initially, and autoregulated 50% in 5.3 ± 6.3 s and 3.5 ± 1.8 s, respectively. Final autoregulation of CPBF and CBF occured in 13.8 ± 18.4 and 16.7 ± 8.8 s.

Our findings indicate that CPBF autoregulates rapidly during moderate increases in MAP.

656.6

COMPARISON OF THE ONTOGENY OF BRAIN OXIDATIVE METABOLISM *IN VIVO* VS *IN VITRO*: AN NMR SPECTROSCOPY STUDY E. J. Novotny, Jr., C. Ariyan, Y. Akiyama*, J.P. O'Reilly, K.L. Behar, G.G. Haddad Depts of Pediatrics and Neurology, Yale Univ., Sch. of Med., New Haven,CT 06510

It has been shown by indirect measurements that oxidative metabolism of brain glucose increases during development. Because the high chemical specificity of NMR spectroscopy permits direct determination of particular metabolic fluxes, we chose to study brain oxidative metabolism in the rat. We also chose to determine whether we can study such questions not only *in vivo* but also in slices in order to probe at mechanisms. A comparison of neocortical glucose oxidative metabolism between slices (400-micron thick) and *in vivo* brain tissue was therefore performed in 10 d vs. 30 d rats using 1-^{13}C glucose combined with proton-observe, ^{13}C-edited NMR spectroscopy. The ^{13}C enrichment of specific carbon positions of glutamate were measured at various time points in perchloric acid extracts of frontoparietal cortex from *in vivo* and slice experiments following administration of ^{13}C labeled glucose. Both the rate of ^{13}C label incorporation into and steady-state isotopic fraction of the C4 carbon of glutamate (glu-C4) were significantly smaller in the 10 d vs 30 d rats *in vivo* ($T_{1/2} \simeq$ 22 vs 10 min; FE=0.2 vs 0.3). In contrast, the difference in the rate of label incorporation into glu-C4 was less in the slices of immature than mature brains ($T_{1/2} \simeq$ 28 vs 22 min) and there was no difference in the isotopic fractions at 120 min (FE=0.34 vs. 0.35). Our results show for the first time that glucose oxidation can be measured directly in as little as two 400 micron slices using this technique. Our conclusions so far indicate that: 1) the lower glu-C4 FE of the immature brain compared to the mature one indicates that sources other than glucose can be used for oxidative metabolism and 2) although differences in glucose oxidation between *in vivo* and *in vitro* conditions are present at both ages, our data suggest that glucose oxidation is more closely correlated with connectivity in the mature than immature brain. (Supported by NS32578, HD32573).

656.7

SPATIAL DISTRIBUTION OF LDH ISOFORMS IN RAT BRAIN IN COMPARISON WITH SDH AND INTRACELLULAR pH. K. L. Lauro, W. D. Lust, J. C. LaManna*, Case Western Reserve University and School of Medicine, Cleveland, OH; 44106

As part of a larger study on adaptation to chronic hypobaric (.5 ATM) hypoxia, we measured micro regional succinate dehydrogenase (SDH) and lactate dehydrogenase isoenzymes (total LDH, LDH-M inhibited and LDH-H inhibited) in the normoxic rat brain through histochemical staining. Activity of the mitochondrial SDH and cytosolic LDH-H is indicative of oxidative capacity, while LDH-M is indicative of glycolytic metabolism. Additionally, pH, was measured via neutral red histophotometry to determine whether pH, is indicative of type of metabolism verses level of metabolic activity. Animals were frozen in situ after neutral red infusion. The frozen block face was photographed for pH analysis prior to removing 6 consecutive 20 or 6 micron sections. Tissue stained for LDH was defatted prior to staining.

LDH-M was most prevalent in regions of highest glycogen content, ie. the hippocampal regions of the oriens, radiatum, and astrocytic layer adjacent to the granular layer of the dentate gyrus. The marked exception to this was the oxidative region in the proximal radiatum of CA3.

The oxidative profile depicted by SDH was similar to LDH-H, yet there were distinct differences, possibly indicating as unique functional role for LDH-H. LDH-H was prominent in the pyramidal cell bodies of the hippocampus, yet SDH was nearly absent. The cerebellar purkinje cells had higher amounts of all LDH isoenzymes than the granular cells, but comparable amounts of SDH. While SDH and LDH-M was virtually absent in the corpus callosum, LDH-H was present in the in low amounts. Capillaries stained heavily for LDH-H. In the regions studied, pH, correlated with degree of oxidative metabolism. Regions of high oxidative enzyme activity, are slightly more acidic than the cortex, while regions with high glycolytic capacity tend to be alkaline. The exception to this was the columnar acidic pattern in the glycolytic radiatum, possibly indicative of activation.

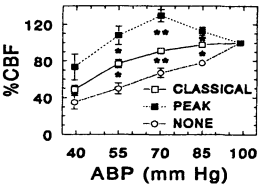
656.8

NON-CLASSICAL PATTERN OF CEREBRAL BLOOD FLOW (CBF)–PRESSURE AUTOREGULATION. S.C. Jones*, C.R. Radinsky, A.D. Perez-Trepichio, Cerebrovascular Res. Lab, Cleveland Clinic Foundation, Cleveland OH 44195.

CBF–pressure autoregulation has been typically characterized by a plateau until the lower limit (LL) is reached, when CBF becomes linearly related to pressure. We have investigated variations in this classical pattern.

Thirty-five Sprague-Dawley rats (389±8 g, SEM) were anesthetized (halothane, N_2O, O_2, body temperature 37°C). The first day a plastic cranial window was implanted and the animal was allowed to recover. The second day, animals were anesthetized, tracheotomized, ventilated, and catheters were placed. Physiologic variables (MABP, $PaCO_2$, PaO_2, and pH) were stabilized. CBF was determined using laser Doppler flowmetry. Animals with low CO_2 and ADP reactivity were excluded. MABP was sequentially lowered by exsanguination to 100, 85, 70, 55, and 40 mm Hg. The LL of autoregulation and patterns were visually identified from the CBF vs MABP plots as either "classical" (n=18), "peak" (n=11) or "none" (n=6). The classical pattern exhibited a plateau followed by a pressure-passive drop. The peak pattern was characterized by a rise in CBF from control as pressure decreased, followed by a fall. No autoregulation (none) consisted of a linear fall of CBF from 100 to 40 mm Hg.

A clear difference between the classical, peak, and none patterns is evident (RM-ANOVA, $p<0.001$; *,**: $p<0.05$, 0.01, Scheffe). The classical and peak lower limits were similar (75±4 and 69±3 mm Hg). The peak pattern has been observed previously by Chen et al (Stroke 15, 343) and indicates that vessels are over-dilating in response to the pressure decrease. Almost a third (31%) exhibited no autoregulatory plateau up to a MABP of 100 mm Hg in spite of acceptable CO_2 and ADP responses. (Support: NSF IBN 90-22190)



656.9

CEREBRAL VASOMOTION: 0.1Hz OSCILLATION IN IMAGING OF NEURAL ACTIVITY. J. E.W. Mayhew, S. Askew, Y. Zheng, J. Porrill, G.W.M. Westby, P. Redgrave, D. M. Rector, R.K. Harper* and R. M. Harper. AIVRU, University of Sheffield, Sheffield, S10 2TP, U.K.; Dept. Anatomy and Cell Biology, UCLA, Los Angeles, CA, 90095-1763, U.S.A.

Reflected light from the surface of neural structures can reveal the functional architecture within large populations of neurons. The signals are derived from multiple sources, including cerebral microcirculation, light scattering properties of active neurons, and possibly glia. We collected image sequences of reflected light at video frame rates (25Hz), using a miniaturized camera and fiber optic probe, and determined that a principal component of intrinsic signals is a modulation of a pervasive low frequency oscillation in the local microvasculature. This oscillation, of approximately 0.1Hz, is present in many structures; the rat cerebral cortex, striatum, thalamus, superior and inferior colliculus, and cerebellum, and cat hippocampus, together with faster (100Hz) and slower components. Although the mechanisms producing the 0.1Hz signal are uncertain, a similar vasomotion oscillation occurs in the peripheral circulation. Concurrent laser doppler flowmeter measures find comparable low frequency signals; however, the image analyses additionally revealed a complex spatio-temporal structure to the 0.1Hz signal, with stimulation-specific patterns. Coherence analyses indicated that the signals cannot be attributed to simple motion artifacts associated with the cardiac or respiratory cycles. Significant regional modulation of the phase structure and amplitude of the 0.1Hz signal emerged in transitions between sleep states, following visual, auditory and noxious stimulation, and following electrical stimulation of the imaged tissue. Reflected light imaging provides a means to assess modulation of microcirculation spatio-temporal dynamics by populations of active neurons. *(Supported by HL-22418, NIDR DE-07212, and Wellcome Trust 038011/Z/93.)*

656.10

CAPILLARY MODULES IN MOUSE CORTICAL BARRELS. J. Sui, C.M. Rovainen*, T.A. Woolsey, Departments of Neurology & Neurological Surgery and Cell Biology & Physiology, Washington University School of Medicine, St. Louis, MO 63110.

A penetrating arteriole which is injected with dye and the surface venules from which dye emerges is an *arteriolar domain* which approximates the area of but does not coincide with a cytochrome oxidase stained barrel in layer IV (Sui et al, '94). To test the hypothesis that an internal terminal arteriole and its capillary "module" is matched to a barrel, adult mice were fixed with mixed aldehydes. Penetrating arterioles and their terminal branches were stained by perfused Rose Bengal. The cortex was cut vertically or tangentially at 200μm with a vibratome and barrels visualized with oblique lighting. In tangential sections 1-2 terminal arterioles supplied each layer IV barrel. Arteriolar branches in layer III were impaled with 3μm glass micropipetes; injected dye was recorded by videomicroscopy progressing from arterioles into capillaries and then venules. Side branches from a vertical arteriole supplied modules of capillaries within the barrels. The capillary modules matched to neural modules, manifest as barrels, are a substrate for control of LCBF to a single cortical column during neural stimulation.

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656.11

WHISKER EVOKED LCBF IN DIFFERENT LAYERS OF RAT BARREL CORTEX. **Yu. Moskalenko, C.M. Rovainen, T.A. Woolsey, D.Liu, V. Sememia, N. Malisheva, W.M. Landau*, Sechenov Institute of/for Evolutionary Physiology and Biochemistry, Russian Academy of Science(s), St.Petersburg, Russia and Departments of Neurology & Neurological Surgery and Cell Biology & Physiology, Washington University School of Medicine, St.Louis, MO 63110.**

Dynamic changes in local cerebral blood flow (LCBF) were monitored simultaneously in cortical layers I-III and IV-V by H_2 clearance. Three Pt electrodes with diameters of 40-60μm and 150-200μm exposed lengths were inserted at 25-30° to the pia in whisker barrel cortex targeted by intrinsic signals in rats anesthetized with 1g/kg urethane. H_2 was generated steadily from the middle electrode; the changes in PH_2 were recorded at the upper and lower electrodes. Resting LCBF levels determined by clearance after H_2 inhalation were 20-30% higher in the middle than superficial layers. Single, rows, or all contralateral whiskers were stimulated mechanically at 3 Hz for 1 min for 5 trials which were averaged. In many cases there was an initial decrease in apparent LCBF. Increases in LCBF up to 30-35% were larger in middle layers. These results are consistent with a model for blood flow regulation in which layer IV afferent stimulation initiates LCBF increases.

Supported by NIH Grants TW 00047, NS 28781 and HL 41075, the McDonnell Center for Studies of Higher Brain Function and an award from the Spastic Paralysis Foundation of the Illinois-Eastern Iowa District of the Kiwanis International.

656.12

SYNCHRONIZATION OF EEG ACTIVITY IN CEREBRAL CORTEX BY STIMULATION OF NUCLEUS TRACTUS SOLITARII (NTS) IN RAT: COUPLING TO CEREBRAL BLOOD FLOW AND MEDIATION BY ROSTRAL VENTROLATERAL MEDULLA (RVL). E.V. Golanov* & D.J. Reis. Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

Electrical stimulation of NTS in cat synchronizes EEG activity (Magnes et al., *Arch. Ital. Biol.* 99:33, 1961). We investigated whether NTS stimulation in rat elicits a comparable response, if it is mediated through RVL and is coupled to the changes in cerebral blood flow (rCBF) mediated by that nucleus (Golanov & Reis, *J. Cereb. Blood Flow Metab.* 14:492, 1994). Rats were anesthetized, spinalized and ventilated while monitoring electrocorticographic activity (ECoG). rCBF was recorded by laser-Doppler flowmetry. Electrical stimulation of NTS (50 Hz, 5 sec, 50 mA) globally synchronized ECoG activity within 1 sec and increased, for 30-60 sec, the powers of the 4-5 Hz and 8 Hz components by >100% ($p<0.01$) (as assessed by fast-Fourier transformation, FFT). rCBF was increased within 1 sec, reached maximum (8±1%; $p<0.05$) by 4-5 sec and declined with ECoG recovery. The stimulus-response characteristics of ECoG and rCBF were comparable (optimal frequency at ~50 Hz and threshold currents at ~20 mA) with active sites located in commissural NTS. Electrical or chemical stimulation of RVL elicited comparable but more potent effects: ECoG synchronization was accompanied by increases in the 4-6 Hz and decreases in the 1-3 Hz-components ($p<0.01$) and rCBF was elevated by 18±3% ($p<0.01$). The active zone in RVL was caudal to the sympathoexcitatory region and bilateral electrolytic lesions therein blocked the synchronization of ECoG and elevation of rCBF elicited from NTS. We conclude: (a) excitation of commissural NTS synchronizes the ECoG and coordinately elevates rCBF; (b) the responses result from excitation of neurons in a subzone of RVL; (c) the pathway may be relevant to some behaviors elicited by natural stimulations of visceral afferents and intrinsic neurons of RVL.

656.13

PRACTICAL INTRACRANIAL ANEURYSM HEMODYNAMICS D.J. Vincent* and J.A. Horton. Department of Radiology, Med. Univ. of S. Carloina, Charleston, SC 29425

To identify the basic underlying principles governing aneurysm hemodynamics, we examined flow patterns in viscoelastic aneurysm models. A pulsatile pump provided realistic flow, and injected food coloring marked flow streams. Each model has several aneurysms in series, and they differ by a single variable--either diameter of aneurysm or relative width of neck. This permitted us to make direct comparisons. We found that angular flow velocity varies inversely with aneurysm diameter; we also found that in aneurysms of similar size, the one with the wider neck will have more rapid angular flow. These results suggest roughly quantitative rules that govern the speed and turbulence of flow within aneurysms. They also have implications regarding aneurysm hemorrhage and growth.

656.14

REGIONAL CEREBRAL DEOXYGLUCOSE UPTAKE IN PREWEANLING RATS UNDER CONDITIONS OF BEHAVIORAL ACTIVATION. M. W. Lilliquist*, F. Gonzalez-Lima, & A. Amsel. Dept. of Psychology & Institute for Neuroscience, University of Texas at Austin, Austin, TX 78712.

A previous report of regional deoxyglucose (2DG) autoradiography in freely moving rats (Nehlig et al., 1988) revealed a developmental pattern from low and uniform rates of 2DG utilization in preweanling animals to higher and more heterogeneous rates in adult animals. Importantly, animals in this previous study were maintained in a small holding box throughout the 2DG uptake period. We report ^{14}C(U)-fluorodeoxyglucose (FDG) uptake values in preweanling animals under conditions of behavioral activation: appetitive learning in a straight-alley runway under different schedules of reinforcement. Under these conditions, we find significant heterogeneity of FDG uptake, revealing far greater differentiation of metabolic indices from low-activity regions to high activity regions. Methodological differences between the present and previous studies, which may contribute to the different findings, will be presented. It is suggested, however, that the relationship between regional cerebral metabolic activity and behavioral maturation may be better reflected under conditions of behavioral activation. Similar findings concerning resting vs. active state in human brain imaging studies will be discussed. (Supported by NIH grants RO1MH43353 and AA07052.)

656.15

POTENTIATION OF INTERSTITIAL ADENOSINE WITH NITROBENZYLTHIOINOSINE (NBTI) AMERLIORATES DELAYED CORTICAL HYPOPERFUSION FOLLOWING GLOBAL CEREBRAL ISCHEMIA IN THE PIGLET. Y.B. Kim, T.S. Park, E.R. Gonzales, A.R. Shah, M.J. Noetzel*, J.M. Gidday. Departments of Neurology and Neurological Surgery, Washington University School of Medicine, St. Louis, MO 63110

A period of delayed hypoperfusion characterizes reperfusion (RP) following transient global cerebral ischemia. Concomitantly, reactivity of the cerebral vasculature to many physiologic and pharmacologic stimuli is impaired. We tested the hypothesis that increases in cerebral blood flow (CBF) during RP could be realized by augmenting endogenous adenosine (ADO), a potent cerebral vasodilator. Microdialysis probes containing platinum wires for measurement of local CBF by hydrogen clearance were inserted bilaterally into the cortex of 9 isoflurane-anesthetized newborn pigs. One probe was perfused with the ADO transport inhibitor NBTI (100μM in mock CSF at 2 μl/min), the other with mock CSF alone. Measurements of [ADO] (μM) and CBF (ml/min/100g) were determined at 20-min intervals during RP following 10-min of global cerebral ischemia induced by ligation of brachiocephalic and subclavian arteries. The table shows that both ADO and CBF were reduced during post-ischemic RP, but [ADO] elevation by NBTI lessened the extent of delayed hypoperfusion. (*p<0.05 vs baseline; †p<0.05 vs control side).

	Baseline	20-40 min RP	40-60 min RP
[ADO] (control)	1.44±0.17	0.83±0.11 *	0.74±0.08 *
[ADO] (NBTI)	3.45±0.67 †	2.67±0.74 †	1.89±0.24 *†
CBF (control)	39±3	19±3 * (-51%)	22±2 * (-42%)
CBF (NBTI)	60±4 †	43±3 *† (-28%)	45±3 *† (-24%)

These results suggest that adenosine can improve post-ischemic tissue perfusion, and, in so doing, may contribute to its neuroprotective effect.

656.16

ELEVATION OF CYTOKINES IN THE CSF OF THE PATIENT WITH VASOSPASM AFTER SUBARACHNOID HEMORRHAGE. M Suzuki*, T Mase, M Sohma, K Miura, M Doi, A Kudo, N Kubo, K Kuroda, A Ogawa, S Endo and K Inada Depts Neurosurg, Bacteriology, and Crit care & Emreg Center, Iwate Med. Univ., Morioka 020 Japan

It has been focused that inflammation occurs in the CSF space after subarachnoid hemorrhage (SAH), and causes delayed vasospasm (VS). To clarify the detail of this mechanism, we analyzed cytokines (TNF, IL-6, IL-8, IL-1β, IL-1 RA) and soluble component of the adhesion molecules (ICAM-1, VCAM-1, ELAM-1) in the CSF and blood. Clinical materials were ten patients of severe SAH. CSF and blood were obtained 3 times simultaneously (day 3-5, 7-9, 12-14) after the onset. VS was diagnosed with angiography and neurological symptoms.

IL-6, IL-8, IL-1β, IL-1 RA were significantly higher in the CSF than in the blood ($p<0.05$). IL-6 and IL-1 RA was higher in the CSF of the patients with VS ($p<0.05$) during day 7-9 than the VS negative patients. Besides, IL-1 RA was also higher in the blood of the VS patients in the same period ($p<0.05$). The value of the adhesion molecules were significantly higher in the blood than in the CSF at any sampling points, but no relation was found to VS. Unique behavior of cytokines, especially IL-6 and IL-1RA, indicates the presence of specific inflammation in the CSF milieu. Elevation of the adhesion molecules in the blood may not contribute provocation of the inflammation in the CSF. Although the sources of IL-6 and IL-1 RA in the CSF are still unknown, understanding of the initiation of the inflammation in the CSF following SAH may open the therapeutic window for the prevention of VS.

656.17

ANGIOGENESIS ACCOMPANIES MAST CELL PROLIFERATION IN EXPERIMENTAL NEUROMAS. **C. Nguyen, T.E. Feasby*, D.W. Zochodne University of Calgary, Calgary, Canada T2N 4N1**

We studied changes in local blood flow, mast cells and blood vessel anatomy in experimental sciatic nerve 'neuromas' of rats (created by sectioning of the midsciatic nerve and resection of the distal nerve and its branches to prevent regeneration). In previous work, we reported that rises in blood flow of early (48 hour) sciatic neuromas were mediated by local CGRP (calcitonin gene-related peptide) accumulation with vasodilation whereas persistent rises in blood flow at later time points (14 days) were not associated with CGRP. In this work, we studied the vascular anatomy of experimental neuromas by infusing, through a femoral catheter, anaesthetized rats with gelatinized India ink prior to euthanasia. Neuromas and intact contralateral nerves were then removed, frozen and sectioned unfixed. Numbers and areas of individual perfused vessels in transverse section were measured using a computerized image analysis setup. Starting at 7 days following neuroma creation, multiple thin walled perfused microvessels were noted, most prominently in the loose connective tissue around the outside of the neuroma. The size and multiplicity of these perfused vessels indicated angiogenesis with vascular proliferation. Similar findings were not observed in contralateral intact nerves, sham-exposed nerves, or 'neuromas' at earlier time points. These microvascular changes paralleled rises in transverse nerve area, and mast cell numbers, density and degranulation from previous work.

Persistent rises in local blood flow of experimental neuromas are associated with a proliferative cellular response involving microvessels, mast cells and likely other tissue elements.

656.18

•LOCAL CEREBRAL GLUCOSE USE IN RETINOFUGAL TARGETS AFTER PARTIAL AND TOTAL RETINAL DEAFFERENTATION: A 2-DG STUDY. •U.Schmitt†, B.A. Sabel†, R. Cross♦, F.E. Samson♦ and T. Pazdernik†* †Inst. Med. Psychol., Univ. Magdeburg, Germany. ♦R.L. Smith Res. Cent. Univ. Kansas Med. Ctr., Kansas City, KS 66160.

• Rats given an optic nerve crush partially regain metabolic activity in retinofugal targets despite a 90% loss of retinal ganglion cells. Thus, there is either some restoration of information transfer from retina to brain or there is an adaptation such that visual function occurs with less information transfer. The 2-[^{14}C]deoxy-glucose technique was used to determine local cerebral glucose use (LCGU) as an assessment of information transfer from retina to brain visual centers. Male adult Long Evans rats were given a mild crush (6/group) or cut (3/group) to the right optic nerve and LCGU was assessed 2, 9 or 8 (cut) days later. The LCGU procedure was determined during stimulation with a flasing strobe-light and rotating bar pattern with or without physostigmine (300μg/Kg; i.m.), a cholinesterase inhibitor known to activate retinocollicular pathways. Quantitative analysis of LCGU autoradiograms according to the Sokoloff equation from rats 2 days after crush revealed a marked reduction in LCGU in all retinofugal targets. There was some restoration of LCGU at 9 days after crush and at this time there was not a statistically significant difference from values 8 days after the cut. Physostigmine treatment enhanced suppression of retinal driven metabolic activity and, therefore, the increase in glucose metabolism between day 2 and 9 was greater. These results provide evidence that LCGU in retinfugal targets is partially restored due to relief of an early lesion dependent depression of neuronal activity (Diaschisis).

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656.19

ISCHEMIA ALTERS CEREBROVASCULAR K^+ CHANNEL FUNCTION IN PIGLETS. D. W. Busija*, and T. M. Louis. Dept. of Physiol./Pharmacol., Bowman Gray School of Medicine, Winston-Salem, NC 27157, and Dept. of Anat./Cell Biology, East Carolina University, Greenville, NC 27858.

Previous studies have shown that ischemia alters ion channel function in cortical neurons in piglets (AJP: 265; H389). The purpose of this study was to examine the effects of ischemia on ATP-sensitive K^+ channels in cerebral arterioles. In anesthetized piglets equipped with cranial windows, we examined arteriolar responses to aprikalim (APK) (an ATP-sensitive K^+ channel agonist) prior to and following 10 minutes of total global ischemia. Ischemia was induced by increasing intracranial pressure. Under control conditions, APK dilated arterioles by $7\pm1\%$ and $24\pm4\%$ at 10^{-8}M and 10^{-6}M, respectively (n=6). Coapplication of glibenclamide (10^{-5}M; n=9) blocked dilation to APK. Following ischemia (n=6), arteriolar dilation to 10^{-6}M APK was reduced to $8\pm4\%$ ($P<0.05$) at 1 hr and $16\pm3\%$ ($P<0.05$) at 2 hrs post-ischemia. In other animals, pretreatment with indomethacin (5mg/kg, iv), which blocks superoxide anion production in cortex, prevented attenuation by ischemia of arteriolar dilation to APK. We conclude that ischemia alters function of vascular K^+ channels probably via effects of oxygen radicals. Supported by HL 30260, HL 46558, & HL 50587.

656.20

DECREASED SENSITIVITY OF CEREBRAL CELLULAR ENERGY STATE TO HYPOXIA IN MICE FED A CREATINE ANALOGUE. D Holtzman*, R Meyers, E O'Gorman, T Wallimann. Children's Hospital, Boston, MA and Swiss Federal Inst. of Technology (ETH), Zurich, Switzerland.

The maturational increase in brain creatine kinase (CK) catalyzed reaction rate in vivo is temporally associated with the appearance of close coupling of the phosphocreatine (PCr) and ATP losses in the hypoxic mouse or rat brain (Holtzman et al., Dev. Neurosci., 1993;15: 261-270). Feeding the creatine transport inhibitor, B-guanidinopropionic acid (GPA), reduces brain CK catalyzed fluxes to the rates seen in the immature mouse (Holtzman et al., Brain Res 1989 483: 68-77) and doubles the brain cytosolic CK isozyme. Therefore, brain CK catalyzed reaction rates and isoenzyme activities were measured in mice fed GPA (2% by weight in standard chow) for at least three weeks starting at age 35 days. Controls received the chow without GPA. 31P NMR spectra and saturation transfer measures of CK catalyzed flux from PCr to ATP were acquired before, during, and after 7 min of hypoxia (6%, 4%, or 2% inspired O2). Brain PCr/nucleoside triphosphate (NTP) ratios and pH values were the same in control and GPA fed mice. Brain CK catalyzed reaction rate constants were about 40% lower in GPA fed mice than in controls ($p<.05$). Total brain CK activity doubled in mice fed GPA for 12 weeks. At each degree of hypoxia, deaths were higher in control than in GPA fed mice (6% O2, no deaths; 4%, GPA 0/6 died, controls 2/6 died; 2%, GPA 1/6 died, controls 3/6 died). AT 4% O2 GPA fed mice showed no losses of PCr or NTP while surviving control animals showed 40-60% losses of PCr. In conclusion, feeding GPA reduces sensitivity to hypoxic death and to losses of brain high energy phosphates. Two hypotheses for these effects are: (1) the additional phosphagen represented by PCr plus GPAP provides more effective coupling of+ ATP synthesis and demand or (2) metabolic adaptations to the Cr analogue also is adaptive to hypoxia. (Supported by NINDS NS26371).

656.21

RELATIONSHIP BETWEEN FUNCTIONAL AND STRUCTURAL ABNORMALITIES IN GERTSMANN-STRAEUSSLER-SCHEINKER DISEASE, INDIANA KINDRED (GSS-IK). G. D.Hutchins, M.R. Farlow, S. R. Dlouhy, P. Piccardo, B. Ghetti*, Indiana University School of Medicine, Indianapolis, IN 46202

The relationship between functional and neuropathologic abnormalities in a patient with GSS-IK and a mutation at codon 198 of *PRNP* was studied. The patient was a 60 year old female with a twelve year history of progressive dementia, ataxia and Parkinsonism. A quantitative Positron Emission Tomographic (PET) study of brain glucose metabolism was performed, using 2-[F-18]Fluoro-2-Deoxy-D-Glucose (FDG), seven weeks prior to the patient's death. For neuropathologic studies, serial sections of the left cerebral and cerebellar hemispheres were immunolabeled with antibodies to prion protein (PrP) and Tau protein to assess PrP and PrP-amyloid deposits as well as presence of neurofibrillary tangles. Serial 10 µm thick sections were stained with haematoxylin-eosin and automatically aligned (using a 2-D registration algorithm) with adjacent 10 µm anti-PrP immunostained sections. The ratio of the area of immunostained PrP (exceeding 25% threshold of the maximum PrP accumulation in a brain slice) to the area of the cortical or subcortical structures determined on the H&E stained slices was used as an index of PrP deposition. Glucose metabolism was dramatically decreased relative to control subjects throughout the brain. The highest metabolic rates were observed in the calcarine cortex and basal ganglia (4.6 and 4.3 mg/100g/min, respectively). The remainder of the cortical structures and cerebellum had uniformly depressed metabolic rates (range 3.0-3.5 mg/100g/min). The PrP index was highest in the basal ganglia (PrP index range 0.60-0.99) and frontal and parietal lobes (PrP index range 0.4-0.74) with lower levels of accumulation in the temporal and occipital lobes (PrP index range 0.08-0.45). All metabolically impaired regions of the brain demonstrate severe PrP and PrP-amyloid deposits, suggesting a relationship between the functional and structural abnormalities. (Supported by PHS Grant NS 29822).

MOTIVATION AND EMOTION: BIOCHEMISTRY AND PHARMACOLOGY

657.1

CLOMIPRIMINE PRETREATMENT BLOCKS THE DEVELOPMENT OF SCHEDULE-INDUCED POLYDIPSIA IN RATS. B. Knutson and J. Panksepp*. Department of Psychology, Bowling Green Univ., Bowling Green, OH 43402.

In schedule-induced polydipsia (SIP), hungry rats drink excessive amounts of water when administered small food pellets on a fixed interval schedule. Because of its' strong motivational component and excessive nature, SIP has been proposed as an animal model of obsessive-compulsive disorder (OCD). In Experiment 1, we tested whether acute administration of the antiobsessional drug clomiprimine (CMI) could block the development of SIP over seven days. Two experimental groups of rats participated in daily SIP trials, during which they received 60 mg Noyes pellets at fixed intervals of 180 s over the course of an hour. A third control group did not undergo the SIP procedure, but their water intake was monitored. The first experimental group (n = 6) received an intraparietal injection of 5 mg/kg CMI 30 minutes prior to each SIP trial (the CMI-SIP group), while the second experimental group (n = 6) received an injection of vehicle 30 minutes prior to each SIP trial (the VEH-SIP group). The control group also recceived an injection of VEH 30 minutes prior to the hour-long measurement period (the VEH-CONT group). Water intake of all groups during the experimental procedure was measured. While the VEH-SIP group consumed increasing quantities of water over the course of the week, the CMI-SIP group remained at the same low levels of water intake as the VEH-CONT group. In a follow-up experiment two weeks later, the CMI-SIP and VEH-SIP groups participated in fourteen more days of the SIP procedure, this time without any pharmacological pretreatment. The CMI-SIP group took a full 10 days for drinking levels to rise to that of the VEH-SIP group. We are presently analyzing SIP as a function of central serotonin. These findings implicate a serotonergic mechanism in compulsive behaviors and suggest that CMI may prove a viable alternative to neuroleptics in the treatment of polydipsia secondary to schizophrenia in humans.

657.2

DISSOCIATIONS IN HIPPOCAMPAL 5-HYDROXYTRYPTAMINE RELEASE FOLLOWING PAVLOVIAN AVERSIVE CONDITIONING TO DISCRETE AND CONTEXTUAL STIMULI. L.S. Wilkinson, T. Humby, A.S. Killcross, T.W. Robbins and B.J. Everitt. **SPON: Brain Research Association.** Department of Experimental Psychology, University of Cambridge, Cambridge CB2 3EB, U.K.

The experiments examined the release of 5-hydroxytryptamine (5HT) in the hippocampus of freely-moving rats following pavlovian aversive conditioning to discrete and contextual stimuli. Differential conditioning was achieved by manipulating the interval between the offset of a discrete auditory "clicker" stimulus (CS+) and the onset of a footshock reinforcer. Footshock occurred either simultaneously with the last second of the CS+ (in short trace subjects) or 60 seconds later (long trace subjects). In this way, subjects were conditioned to the discrete stimulus or background "contextual" stimuli, respectively. During conditioning subjects also received two unpaired visual stimuli (CS-). At test, dialysates were collected as all animals experienced: (i) the aversive and two other "neutral" environments and (ii) the CS- and CS+, presented in both aversive and neutral environments. Exposure to the aversive (but not either of the two neutral environments) increased hippocampal 5HT release in both short and long-trace subjects, but to a significantly greater extent in the latter. In contrast, hippocampal 5HT release was unaffected by presentation of the CS+ or CS- under all conditions. These data do not agree with the hypothesis that aversive cues generally activate 5HT function. Instead, they suggest a degree of specificity whereby 5HT afferents terminating in particular target structures are differentially activated depending on the nature of, and the extent of conditioning to, the aversive stimulus - in the case of the hippocampus, by contextual and not discrete cues predictive of aversion.

657.3

INTRA-LATERAL HYPOTHALAMIC INJECTIONS OF LOW DOSES OF KAINIC ACID ELICIT LOCOMOTOR ACTIVITY. M.R. Pitzer* and D. Wirtshafter. Department of Psychology, University of Illinois at Chicago, Chicago, Il 60607.

Injections of L-glutamate into the lateral hypothalamus (LH) has been shown to elicit coordinated locomotor activity in the rat. The present study assessed the efficacy of infusing low doses of the L-glutamate analogue kainic acid (KA) into the LH of the awake freely moving adult rat.

Bilateral injections of stimulatory doses of KA (0, 5, 10, 20 or 40ng/0.5ul) produced dose dependent increases in locomotor activity which lasted for more than an hour. Moreover, locomotor activity counts, as measured in locomotor photocell boxes, did not differ when KA was injected at one of three separate rostro-caudal LH levels.

These results, which are similar to those following L-glutamate injections, suggest that the activation of KA receptors throughout the rostro-caudal extent of the LH is capable of inducing hypermotility in a dose-dependent fashion in the awake adult rat.

657.4

THE ROLE OF GALANIN IN THE VENTRAL TEGMENTUM FOR THE EXPRESSION OF DEPRESSION. M.K. Demetrikopoulos*, A. J. Turner, R.W. Bonsall, C.H.K. West, & J.M. Weiss. Dept. of Psychiatry, Emory University, Atlanta, GA 30306.

Burst firing of locus coeruleus (LC) neurons, as occurs during stress, causes release of galanin from terminals in the ventral tegmental area (VTA). It is hypothesized that galanin plays a role in depressive symptomatology by inhibiting activity of VTA dopaminergic (DA) neurons. The present study examined motor activity in a novel environment and a swim test following galanin infusion into the VTA. Experiment 1 utilized male Sprague-Dawley rats which had been selectively bred to struggle during a swim test. Awake subjects received a bilateral infusion (3.0 µg in 3.0 µl/side over 6 min) of galanin, heat-inactivated galanin, or artificial CSF vehicle. Following infusion, subjects were placed in a novel environment (plexiglas box 16 x 20 in) equipped with infrared beam sensors which recorded spontaneous locomotor activity and rearing for 40 min. Galanin reduced ambulation ($p<.01$) and rearing behavior ($p<.01$) compared to controls. Immediately following the exploratory task, subjects were exposed to a 15 min swim test. Galanin did not affect struggling activity of experimentally naive subjects, but reduced struggling in a sub-set of the subjects that had been swim-tested previously ($p<.01$). Experiment 2 tested non-struggle-selected male Sprague-Dawley rats in the swim-test immediately following galanin infusion. Infusion of galanin into VTA resulted in increased floating behavior in these subjects ($p<.04$). Overall, these data suggest that galanin infusion into VTA results in decreased motor activity and suggest a mechanism for mediation of depressive symptomatology through interaction of the LC with the VTA DA system.

657.5

ACTIVATION OF CHOLINERGIC CELLS IN THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS PRODUCES AVERSION, NOT REWARD. R. Mehta and K.B.J. Franklin* Department of Psychology, McGill University, Montreal, Quebec, Canada, H3A 1B1.

The pedunculopontine tegmental nucleus (PPTg) has been found to play a critical role in the rewarding effects of morphine and amphetamine, as demonstrated in the conditioned place preference (CPP) paradigm. This nucleus is a major source of cholinergic projections to the substantia nigra and is believed to mediate activity of dopamine neurons. The firing rate of cholinergic neurons in the PPTg is regulated by autoreceptors that can be blocked by muscarinic antagonists to increase cell firing. Thus, microinjection of muscarinic antagonists into the PPTg elicits behaviours characteristic of dopamine cell activity, such as circling. If the role of the PPTg in reward is to modulate dopamine neurons involved in reward, a muscarinic antagonist injected into the PPTg should be rewarding. To test this hypothesis, scopolamine methyl bromide (5 µg/µL to 20 µg/µL) or 0.9% saline, intra-cranially injected into the PPTg, were used as reward treatments in a balanced CPP paradigm (3 pairings). On the test day, rats treated with scopolamine methyl bromide showed a significant conditioned place aversion, suggesting that activation of cholinergic cells in the PPTg is not involved in reward.

657.6

THE NEUROCHEMICAL ORGANIZATION OF EMOTIONAL TOLERANCE IN AN AVIAN MODEL.

G. Bernroider *, M. Waldhör and B. Minnich, Institute for Zoology, University of Salzburg, Austria

Various forms of emotionally guided learning behaviour carry an intense steroidal and peptidergic-opioid component. In addition, evidence from chronic opiate application studies suggests a behavioural equivalence to some neural mechanisms inducing states of tolerance and dependence. Using a precocial avian model (naive, day-old quail chicks), we show that visually guided social learning is reflected by a gradual return of aversive behavioural reactions such as distress vocalizations, to a pre-disposed level of discomfort in course of chronic 'comforting' exposures. This parallels neural mechanisms underlaying the acquisition of tolerance to chronic drug exposure. Acute 'withdrawal effects' are behaviourally reflected by isolation induced protest vocalizations (DV) following social isolation. These findings are accompanied by a close association of opiate receptor organization with levels of behavioural expressions. [^{3}H]-etorphin binding to quail brainstem tissue is identified as the neural substrate responsive to steroid driven organizational differences in the expression of deprivation effects. In addition, the major noradrenergic sources such as locus coeruleus neurons, show congruent changes in NA turnover rates with respect to different levels of behavioural tolerance. Our model of early social learning addresses substrates of psychotropic drug addiction as adaptive components within a system facilitating social orientation.

657.7

MESOLIMBIC DOPAMINERGIC NEURONS AND AFFECTIVE PERCEPTION : INVOLVEMENT OF AMYGDALA- NUCLEUS ACCUMBENS INTERACTIONS. A. Louilot* and C. Besson. INSERM U. 259 - Université de Bordeaux II - Domaine de Carreire - 33077 Bordeaux Cedex - France.

We demonstrated previously that the responses of mesolimbic dopaminergic (DAergic) neurons to an appetitive or to an aversive stimulus are opposite in the left *core* part of the nucleus accumbens (ACC). Anatomical and functional relationships between the amygdala and the ventral striatum have been reported. In the present work, we tested the hypothesis that the DA responses to the affective value of a stimulus are dependent on the basolateral nucleus of the amygdala (BLA).

The DAergic reactivity was studied using the voltammetric measurements of DA in the ACC of freely moving male rats. Selective detection of the DA signal was obtained by the computed numerical analysis of the catechol signal. Experimental procedure was as following: animals were placed during one hour in the experimental cage; they were thus exposed for one hour to an appetitive olfactory stimulus (banana) and received consecutively either an injection of saline (NaCl 0.9%) (control group) or an injection of LiCl (0.15M) (experimental group) and stayed one more hour in the experimental cage; 72 h later animals were again exposed for one hour to the conditional olfactory stimulus (CS). Two hours before the second exposition to the CS animals received either a microinjection of phosphate buffered saline (PBS) or a microinjection of tetrodotoxin (TTX) in BLA.

The following results were obtained : during the 2nd presentation of the CS an increase of about 120% above the baseline was observed in the control group+PBS whereas DA signal decreased rapidly of about 25% in the experimental group+PBS; in the control group+TTX no significant change in DA release was observed whereas in the experimental group+TTX the olfactory stimulus induced a rapid increase of about 50% above the basal level.

The results of the present study strongly suggest that BLA is directly involved in the perception of stimulus presenting an appetitive value and that other structures interact with the BLA for the perception of the stimulus as aversive.

657.8

BLOCKADE OF NON-NMDA RECEPTORS IN THE NUCLEUS ACCUMBENS SHELL AND VENTROMEDIAL STRIATUM ELICITS INTENSE FEEDING IN RATS. C.S. Maldonado-Irizarry*, C.J. Swanson, A.E. KELLEY. Dept. of Psychiatry, University of Wisconsin-Madison Medical School, Madison, WI 53705

The present studies investigated the role of glutamatergic inputs within the ventromedial striatum in ingestive behavior. Local microinfusion of the AMPA/kainate antagonists DNQX, CNQX and NBQX (0, 0.25, 0.75 µg bilaterally) into the shell subregion of accumbens resulted in an immediate, pronounced feeding response (in sated rats) that was dose-dependent. Infusion of equimolar doses of DNQX into the core subregion, 0.8 mm lateral to the shell site, did not induce feeding. The feeding response proved to be pharmacologically selective in that infusion of NMDA antagonists AP-5 and MK-801 into the accumbens shell did not affect feeding behavior. Systemic pre-treatment with naloxone did not affect the feeding response to DNQX infusion into the shell; however, feeding was reduced upon systemic administration of D1 and D2 antagonists. Furthermore, concurrent injection of the GABA agonist muscimol into the lateral hypothalamus, a major target of shell efferents, abolished the response. A mapping study of further striatal sites indicated that no feeding was elicited from ventrolateral, posterior dorsal, anterior dorsal, and dorsomedial sites following DNQX infusion. However, a significant feeding response was obtained from the ventromedial caudate, just posterior to the accumbens. The most sensitive site thus far examined was the posterior accumbens shell. Animals infused with a low dose of DNQX (50 ng) into this region showed a striking feeding response. These data suggest that AMPA/kainate receptors in the accumbens shell, and perhaps neighboring ventromedial striatal regions, play a critical role in ingestive behavior, perhaps via the lateral hypothalamus.

657.9

NMDA ANTAGONIST AP5 BLOCKS THE ANXIOGENIC EFFECT AND LACTATE INDUCED PHYSIOLOGICAL AROUSAL CAUSED BY CHRONIC GABA DYSFUNCTION IN THE DORSOMEDIAL HYPOTHALAMUS OF RATS. A.Shekhar* and S.R.Keim Dept. of Psychiatry, Indiana Univ. Med. Center, Indianapolis, Indiana 46202.

The present study was conducted to test if the panic-like state caused by chronic dysfunction of GABA inhibition in the dorsomedial hypothalamus (DMH) is due in part to increased N-methyl-D-aspartate (NMDA) receptor mediated excitatory tone and therefore can be blocked by acute injection of the NMDA antagonist AP5. Rats were fitted with femoral arterial and venous catheters and baseline response in the social interaction (SI) test of anxiety as well as heart rate (HR) and blood pressure (BP) responses to sodium lactate infusions (10 ml/Kg i.v.) were obtained. Unilateral, chronic injection cannulae connected to a detachable Alzet infusion pump filled with the GABA synthesis inhibitor l-allylglycine (l-AG; 3.5 nmoles/0.5ul/hr) were implanted into the DMH. After 4 days, rats were tested in SI and with i.v. lactate infusions to establish the development of anxiety and increased HR and BP responses to lactate. On days 6 and 7 of l-AG infusions into the DMH, after injection of either artificial cerebrospinal fluid (a-CSF) or the NMDA antagonist AP5 (100 pmoles/ 100 nl) into l-AG infusion site in the DMH in random order, rats were once again tested in SI and with i.v. lactate infusions. Injecting AP5 and not a-CSF into the DMH significantly blocked the chronic GABA dysfunction induced anxiogenic effect and lactate response, suggesting an increased NMDA mediated excitatory neurotransmission in this region (Supported by MH 45362).

657.10

MUSCARINIC RECEPTORS IN THE NUCLEUS ACCUMBENS MEDIATE A REINFORCING EFFECT. S. Ikemoto*, B. S. Glazier, J. M. Murphy, & W. J. McBride. Dept. of Psychiatry, Inst. of Psychiatric Res., Indiana Univ. Sch. of Med. and Dept. of Psychology, Purdue Sch. of Sci., IUPUI, Indianapolis, IN 46202.

The nucleus accumbens (NAC) has been known to be an important brain region for participating in reinforcement processes. Thus far, few studies have examined the possible involvement of the cholinergic NAC system in reinforcement processes. Therefore, the present study was undertaken to determine, using an intracranial self-administration technique, if activation of NAC muscarinic receptors produces reinforcing effects. A unilateral 22-ga guide cannula was stereotaxically implanted in adult female Wistar rats and aimed at the NAC. After at least one week of recovery, animals were given the opportunity to self-administer a carbachol solution (n = 6) or its vehicle (n = 5) into the NAC. The testing chamber was equipped with two levers; the depression of one lever (active) delivered 100 nl of a 3.28 mM carbachol solution or artificial cerebral-spinal fluid (aCSF), while the depression of the other lever (inactive) had no programed consequence. No cue or shaping method was employed. During the 3-hour test period, the animals receiving the carbachol solution had significantly more self-infusions ($p = 0.002$) than did the aCSF group (27.8 ± 3.6 v.s. 10.0 ± 1.6 total infusions for carbachol and vehicle animals, respectively). The number of responses on both the active and inactive levers for the carbachol subjects was significantly higher ($p = 0.03$) than that for vehicle animals. Carbachol subjects responded on the active lever significantly more ($p < 0.001$) than they did on the inactive lever (88.7 ± 15.9 v.s. 48.5 ± 15.4 total responses for active and inactive levers, respectively). Vehicle animals did not discriminate between the two levers (27.4 ± 7.1 v.s. 22.6 ± 6.4 total responses for active and inactive levers, respectively). The present results suggest that activation of muscarinic receptors in the NAC produces a reinforcing effect (AA 09619, AA 08553).

657.11

BOVINE GROWTH HORMONE (bGH) TRANSGENIC MICE DISPLAY INCREASED SPONTANEOUS LOCOMOTOR ACTIVITY AND LOCOMOTOR STIMULATORY RESPONSE TO d-AMPHETAMINE B Söderpalm*, R. Seiberlich, J.A. Engel and J. Törnell. Inst. of Physiol. and Pharmacol., Dept. of Pharmacol., Göteborg University, Medicinaregatan 7, 413 90 Göteborg, Sweden.

Recent clinical and animal data indicate a role for growth hormone (GH) in mechanisms related to anhedonia/hedonia, psychic energy and reward. Thus, GH substitution in GH-deficient patients has marked positive effects on the general well-being, psychic energy and drive. Moreover, GH-deficient patients are less frequent smokers than age-matched controls, whereas bovine growth hormone (bGH) transgenic mice show increased preference for ethanol and nicotine over water in free-choice models.

In the present experiments we have investigated whether bGH transgenes and controls differ in spontaneous locomotor activity (LMA), a behavioral response related to brain dopamine (DA) and reward mechanisms, as well as in LMA response to drugs of abuse known to interfere with brain DA systems.

The mice were tested for LMA in activity boxes once a week for five weeks. When first exposed to the test-apparatus bGH animals displayed significantly more LMA during the entire registration period (1 h). One week later the animals received an acute saline injection before being placed into the apparatus. Again bGH transgenes displayed more LMA, but only during the first 30 minutes of the recording period. At the third test occassion all animals received d-amphetamine (1 mg/kg, i.p.). bGH transgenic mice were significantly more stimulated by d-amphetamine than non-transgenes during the entire test-period (1 h). At the fourth and fifth test occassions the animals received ethanol (2.5 g/kg, i.p.) and nicotine-di-tartrate (0.5 mg/kg, s.c.), respectively, and tendencies for larger LMA responses in the bGH transgenes were observed.

In conclusion, bGH transgenic mice display more spontaneous LMA than non-transgenic controls, and, possibly, also a disturbed habituation process. The finding that bGH mice were also more sensitive to d-amphetamine-induced LMA may suggest that the behavioral differences observed are related to differences in brain DA systems; indicating a hyperresponsiveness of these systems in bGH transgenes. These findings may be of relevance for the reported psychic effects of GH in humans.

657.12

FACILITATED ACQUISITION OF SEXUAL BEHAVIOR IN MALE RATS FOLLOWING *d*-AMPHETAMINE-INDUCED BEHAVIORAL SENSITIZATION Dennis F. Fiorino* and Anthony G. Phillips Dept.of Psychology, University of British Columbia, Vancouver, B.C., V6T 1Z4

Drugs of abuse appear to activate neural systems mediating goal-directed behavior towards natural rewards such as food and sex. The mesocorticolimbic dopamine system plays a key role in this respect and repeated administration of psychostimulants leads to sensitization of this system. The present study investigated the effect of sensitization induced by repeated injections of *d*-amphetamine on subsequent sexual behavior in the male rat tested in a drug-free state.

Male rats were given one injection of *d*-amphetamine (1.5 mg/kg, i.p.) every other day for a total of 10 injections, a regimen sufficient to induce behavioral sensitization as measured by increased stereotypy in response to a fixed dose of *d*-amphetamine. After a three-week post-drug withdrawal period, rats were tested for sexual behavior. On day 1, sensitized rats exhibited facilitated sexual behavior in the presence of a receptive female as indicated by a significantly shorter mount latency (x = 123.9 ± 47.4 sec) as compared to controls (x = 272.0 ± 53.7 sec). Further, based on a set of criteria selected to reflect sexual proficiency (i.e. intromission latency ≤ 10 min; ejaculation latency ≤ 15 min; and post-ejaculatory interval ≤ 10 min), sensitized rats showed facilitated acquisition of sexual behavior, reaching the criteria after only 4 test days compared to 6 test days for control rats.

657.13

SULPIRIDE ATTENUATES CONDITIONED PLACE PREFERENCE FOLLOWING SEXUAL BEHAVIOR IN FEMALE SYRIAN HAMSTERS. R.L. Meisel* and M.A. Ioppa. Dept. of Psychological Sciences, Purdue Univ., West Lafayette, IN 47907.

The ability of the dopamine D_2 antagonist, sulpiride, to prevent the acquisition of a conditioned place preference was examined in female hamsters. Females were treated with one of several doses of sulpiride ip (12, 18, or 24 mg/kg body weight) prior to sexual encounters with a male in a conditioned place preference apparatus. Preliminary results indicate that sulpiride-treated females, like vehicle-treated females, exhibited near maximal levels of sexual behavior (lordosis) throughout the conditioning sessions. Despite showing high levels of lordosis, the sulpiride-treated females did not show evidence of conditioned place preference. Vehicle-treated females replicated our earlier findings of conditioned place preference induced by sexual behavior in female hamsters. These results suggest that conditioned place preference is a useful means for probing the appetitive components of female sexual behavior, and that D_2 receptors are involved in this appetitive process.

This research was supported by a grant from the National Science Foundation (IBN-9412543).

657.14

ROLE OF GLYCINE/NMDA RECEPTORS IN THE DORSAL PERIAQUEDUCTAL GRAY OF RATS UNDER THE INFLUENCE OF ANXIOSELECTIVE DRUGS. **A.P. Carobrez*, and M.M. De-Souza. Depto. de Farmacologia, CCB, UFSC, Florianópolis, SC, 88040-900, Brazil.**

The midbrain periaqueductal gray (PAG) coordinates responses of an animal facing aversive stimulus. Excitatory amino acid (EAA) neurotransmission at the dorsal PAG (DPAG), may mediate distinct behavioral performance in animal models of anxiety. The purpose of this investigation was to explore the effects of DPAG-EAA on the behavioral performance of rats under anxioselective drugs effects. Assessment of anxiety-related behavior was performed in male hooded rats with chronically implanted cannula, aimed to DPAG. Fifteen min after receiving an IP injection (1 ml/kg) of diazepam (DZP; 0.375, 0.75 mg/kg), pentylenetetrazol (PTZ; 15, 30 mg/kg) or vehicle, the rats received a microinjection (0.4 μl) of ACSF, glycine (GLY 80 nmol) or 7-Cl-kynurenic acid (7CIKYN) into DPAG. Five min after receiving both treatments the animals were placed in the elevated plus-maze test for a 5 min observation. In experiment 1, DZP increased ($p<0.05$) both, the %entries (OAE; 60 ± 5.8) and the %time spent (TSO; 36 ± 8.9) on open arms when compared to baseline levels (OAE= 31 ± 8.2; TSO= 6.3 ± 2.0), . These DZP anxiolytic effects were blocked after DPAG microinjection of GLY (OAE= 30 ± 9.7; TSO= 19.8 ± 8.3). In experiment 2, the treatment with PTZ decreased the OAE (10 ± 6.9) and the TSO (2.6 ± 2.0). These PTZ anxiogenic effects were reversed after DPAG microinjection of 7CIKYN (OAE= 53 ± 4.1; TSO= 23 ± 6.6). The results suggest that the behavioral effects of anxioselective compounds depend on baseline glutamatergic DPAG activation. Also these results strengthens the hypothesis of a modulatory role for DPAG/EAA system in anxiety. ***Supported by: CNPq and CAPES***

657.15

COMPARISON OF THE EFFECTS OF D_1 AND D_2 DOPAMINE RECEPTOR ANTAGONISTS ON THE RESPONSE-REINSTATING PROPERTIES OF FOOD REINFORCEMENT. A. L. Chausmer*, C. M. Reals and A. Ettenberg. Behavioral Pharmacology Laboratory, Dept. of Psychology, Univ. of California, Santa Barbara, 93106.

The current experiment examined the ability of selective dopamine D_1 and D_2 receptor antagonists to prevent the reinstatement in operant responding produced by a single reinforced trial in the midst of extinction. Hungry rats were trained to run a straight-alley for food reward during single daily trials. After 12 reinforced trials, the operant was extinguished during consecutive daily non-reinforced trials. Ss were then injected 30 min prior to a single Treatment trial with one of four doses of either the D_2 antagonist, raclopride (0.0, 1.0, 0.5 and 0.25 mg/kg IP) or the D_1 antagonist SCH 39166 (0.0, 0.02, 0.01, 0.005 mg/kg IP). Twenty-four hours later, a Test trial was conducted in an unbaited runway. On Treatment day, a single reinforced trial was sufficient to reinstate operant running 24 hrs later (i.e. on Test day). While raclopride on Treatment day dose-dependently attenuated the response-reinstating effects of food as measured on the next trial/day, interpretation of SCH 39166 data was made difficult because it produced no consistent dose-response effects and it attenuated performance of a motor control group. In a separate experiment, neither drug produced reliable reductions in spontaneous locomotor activity measured 24 hrs post-injection. Together these data suggest a clear role for D_2 receptor subtype in food reinforcement, but the role of the D_1 receptor remains unclear.

657.16

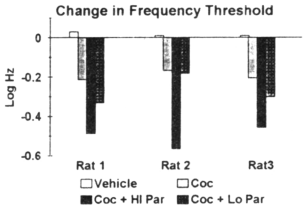
SOCIAL MODULATORY INFLUENCES ON CROWING AND AGGRESSION IN TESTOSTERONE-TREATED CHICKS. L. Normansell* and E. Craven, Program in Neuroscience, Muskingum College, New Concord, OH 43762.

Following chronic testosterone (T) administration, young chicks crow regularly and become very aggressive. In the present series of studies, the ability of varying social contexts to influence these behaviors in T-treated chicks was investigated. Chicks were obtained at one day post hatch, housed in like-treated flocks of 7-9 chicks, and administered either .1ml oil or 2.5mg testosterone in oil per day. When tested at 3-4 weeks of age, in both paired round-robin and group testing with animals from their own flock, T-treated animals were more aggressive and crowed more than control chicks. When who was dominant over whom was determined, the resulting dominance hierarchies were less linear in the T-treated chicks which might indicate a disruption in the development of normal social processes. In paired contests with animals from a different but like-treated flock, T-treated chicks crowed over 3 times more than when they were with an animal from their own flock but did not differ in level of aggression. Next, 2 chicks of one flock were grouped with 2 from a like-treated but different flock. T-treated animals displayed over 7 times as many aggressive acts as controls, but in both groups, these acts were directed equally to the other animals regardless of what group they were from. In this social situation, however, none of the T-treated animals emitted any crows. In summary, while testosterone treatment induces many adult-like patterns of behavior in young chicks, these behaviors are modulated to some extent within the ongoing social environment of the animals.

657.17

THE EFFECT OF SEROTONIN AND DOPAMINE REUPTAKE INHIBITORS ON DORSAL RAPHE SELF-STIMULATION THRESHOLDS. P.Tzvetkov*, S.Khan, E.Miliaressis, G.Fouriezos. University of Ottawa. Ottawa, Canada K1N 6N5.

Male Sprague-Dawley rats were implanted with monopolar electrodes in the dorsal raphe nucleus (DR). Using a descending method of limits, self-stimulation frequency thresholds were obtained before and after acute intraperitoneal injections of cocaine HCl (20mg/kg), paroxetine (10mg/kg), and paroxetine (10 mg/kg or 20 mg/kg) plus cocaine (20 mg/kg). Injection of cocaine, a mixed serotonin/dopamine reuptake inhibitor, led to a 37% drop in frequency threshold. Injection of paroxetine, a selective serotonin reuptake inhibitor, did not decrease threshold. However, paroxetine (20mg/kg) injected 30 min prior to cocaine led to a 69% drop in threshold. Results suggest that increasing serotonin levels may enhance the ability of dopamine agonists to facilitate self-stimulation in the DR.



657.18

DISSOCIATION OF REWARD AND PERFORMANCE CHANGES FOLLOWING ICV MICROINJECTIONS OF NEUROTENSIN. P.-P. Rompré* Département de psychiatrie, Université de Montréal, Montréal (Québec), Canada, H3C 3J7.

Several lines of evidence show that neurotensin (NT) modulates central dopamine (DA) functions. When microinjected into the ventricle, for instance, NT produces neuroleptic-like effects and inhibits the increase in locomotion produced by DA receptor activation in the nucleus accumbens. Such an inhibitory effect of NT on DA-induced locomotion contrasts with its stimulatory effect on DA cell firing and on DA metabolism and release and implies that this postsnyaptic action of NT is strong enough to counteract its action on DA impulse flow. Another possibilty is that the inhibitory effect of ICV NT on DA-induced locomotion results from, or is potentiated by, its non-specific effects as NT is known to induce hypotension, muscle relaxation and hypothermia. In this study, we used the curve-shift method as applied to brain stimulation reward to determine whether the suppressant effect of ICV NT on a DA-dependent behavior is specific or is due to a general decrease in motor capability. Male rats implanted with a stimulating electrode in the central gray and a guide cannula above the lateral ventricle were trained to lever press for trains of 200 msec cathodal pulses of fixed intensity and variable frequency. Once responding was stable, the effects of three doses of ICV NT (3, 10 and 30 µg/10 µl) on the function relating the rate of responding to the stimulation frequency was determined, just before, and for 95 min after the injection. Results obtained demonstrate a clear dissociation between performance and reward changes. At every dose tested, NT produced a significant suppression of maximal responding, reflecting a decrease in motor capability. At the highest dose, a decrease in reward threshold occurred 30 min later than the onset of the suppression of responding and outlasted the later effect by at least 30 min. These results suggest that ICV NT suppresses motor capability, an effect that may interfere with the behavioral expression of an increase in DA-dependent functions. Supported by a grant from the Medical Reseach Council of Canada.

657.19

STRESS-INDUCED SENSITIZATION, BUT NOT FACILITATED LEARNING, IS CHOLINERGICALLY-MEDIATED. **T.J. Shors*, G.P. Mark, J.C. Selcher, and R.J. Servatius. Dept. Psychol. and Prog. in Neurosci., Princeton University, Princeton, NJ 08544.**

Acute exposure to an inescapable stressor of restraint and intermittent tail-shock enhances sensory reactivity and independently facilitates acquisition of the classically conditioned eyeblink response in the freely-moving rat. Because the same stressor increases acetylcholine (ACh) release into the hippocampus, amygdala, and prefrontal cortex, we tested the hypothesis that either one or both of the stress-induced behaviors was cholinergically mediated. Rats (6/group) were treated with the muscarinic antagonist scopolamine (5 mg/kg) or vehicle 30 min prior to the stress and no stress condition. 24 hrs later, they were exposed to 10 white noise stimuli (85 dB) and trained with paired and unpaired stimuli consisting of a white noise conditioned stimulus overlapping and coterminating with a periorbital shock unconditioned stimulus to the eyelid. Preventing activation of muscarinic receptors during the stressor prevented the sensitization ($p = 0.02$) but not the facilitated learning ($p = 0.99$). Additional data suggest that the effective ACh is not originating in the septum; severing the fornix ($n = 12$) did not prevent the enhanced reactivity to auditory cues. These results suggest that the sensitization effect is cholinergically-mediated, although not via septal inputs to the hippocampus. Moreover, these results pharmacologically dissociate the stress-induced sensitization from the facilitation of associative learning. **[Whitehall and McDonnell-Pew Foundations and ONR support to TJS]**

658.1

EFFECTS OF PHASE-SHIFTING TREATMENTS ON THE mRNA LEVELS OF PUTATIVE OSCILLATOR PROTEINS, BiP AND PORIN, IN *APLYSIA*. M. Sloan*, C. Koumenis, and A. Eskin, Dept. of Biochem. & Biophys. Sci., Univ. of Houston, TX 77204.

A circadian pacemaker in the eye of *Aplysia* generates a rhythm of spontaneous nerve impulses. A model of this system predicts that the phase shifting agents light and 5-HT modify the synthesis of oscillator proteins at the level of translation and perhaps transcription as well. Using 2-D gel electrophoresis, we previously searched for putative oscillator proteins in the eye whose synthesis was modified by treatments with light and 5-HT. We found that light treatments to isolated eyes at CT 18-24 increased incorporation of ^{3}H-leucine into BiP (a molecular chaperone in the endoplasmic reticulum), while decreasing incorporation of label into porin (a large conductance channel in mitochondrial and other membranes). 5-HT treatments to eyes at CT 6-12 increased incorporation of label into BiP and porin.

To investigate the effects of phase shifting treatments on BiP and porin at the transcriptional level, we first obtained their cDNAs. Clones of *Aplysia* BiP were obtained from Kuhl, et al., and clones of porin were obtained by screening an *Aplysia* ganglia cDNA library using a clone from human porin. A full-length cDNA of 282 amino acids, obtained from the *Aplysia* library, was 67% identical to human porin. Riboprobes complementary to coding regions of BiP and porin were used in ribonuclease protection assays to measure their mRNA levels. Light and 5-HT administered to isolated eyes had opposite effects on the levels of mRNAs of BiP and porin. The effects of light and 5-HT on BiP and porin mRNAs correlated with their effects on proteins except for the action of light on BiP mRNA and protein. Light at CT 18-24 decreased levels of mRNAs of BiP (-68±2%, n=4) and porin (-67±6%, n=4), while 5-HT at CT 6-12 increased levels of mRNAs of BiP (50±20%, n=7) and porin (45±14%, n=6). These results indicate that light and 5-HT may phase shift the ocular circadian oscillator by regulating levels of BiP and/or porin mRNAs.

658.2

NMDAR1 SPLICE VARIANTS IN THE SUPRACHIASMATIC NUCLEUS (SCN) OF THE SIBERIAN HAMSTER. Francis J P Ebling*, Iona H M Alexander and Jens D Mikkelsen, Dept. Anatomy, University of Cambridge, UK and Institute of Medical Anatomy, The Panum Institute, University of Copenhagen, Denmark.

Glutamate is implicated in mediating the phase-shifting effects of light on circadian rhythms, and our previous studies have revealed the presence of mRNAs encoding glutamate receptors in the rat suprachiasmatic nucleus (NMDAR1/NMDAR2C *Brain Res* 1993, 632: 329-333). The common subunit gene NMDAR1 can be alternatively spliced in three regions to generate functionally-different products, so the aim of the current study was to investigate which mRNA species are present in the SCN. *In situ* hybridization was carried out on series of 12µm coronal sections from the Siberian hamster (*Phodopus sungorus*), using ^{35}S-labelled oligonucleotide probes complementary to common and variable regions of the rat gene (*J Comp Neurol* 1994, 313:1-16, from which terminology is adopted). Probes complementary to VIP mRNA and AVP mRNA defined the ventrolateral and dorsomedial regions of the hamster SCN in adjacent sections. Hybridization of the common probe occurred ubiquitously in the hamster brain, including the SCN. A probe complementary to deletion II/exon 22 was also abundant throughout the SCN. In contrast, no hybridization of a probe complementary to insertion I/exon 5 was detected in the SCN or other hypothalamic nuclei, despite a highly characteristic pattern of expression restricted to the thalamus. Background on sections hybridized with a probe complementary to deletion I/exon 21 was high, but no clear evidence of hybridization in the SCN was obtained. These observations suggest that the predominant isoform in the SCN is NMDAR1C, and provide further evidence that glutamate is an important regulator of SCN function. *[Supported by The Wellcome Trust grant 037667/Z/93 and by The Royal Society]*

658.3

AN ALTERED CIRCADIAN CLOCK IN NCAM-180 DEFICIENT MICE. H. Shen[1], M. Watanabe[2], H. Tomasiewicz[3], U. Rutishauser[3], T. Magnuson[3] & J. D. Glass*[1], [1]Dept. of Biological Sciences, Kent State Univ., Kent, OH 44242, [2]Division of Pediatric Cardiology, Dept. of Pediatrics, Rainbow Babies & Children Hospital, Case Western Reserve Univ., Cleveland, OH 44106, and [3]Dept. of Genetics, Case Western Reserve Univ., Cleveland, OH 44106.

The basis of the circadian pacemaker in the suprachiasmatic nuclei (SCN) remains speculative. Our previous study confirmed robust expression of highly polysialylated neural cell adhesion molecule (PSA-NCAM) in adult rodent SCN. PSA-NCAM, capable of modulating contact-dependent cell-cell interactions, is involved in a variety of developmental events and in adult neuroplasticity. To explore the role of PSA-NCAM in the SCN circadian clock, we assessed circadian locomotor activity rhythms under various photoperiod regimens in the mice with genetic deletion of the major PSA-associated NCAM isoform, NCAM-180. The results showed that NCAM-180 deficient mice (n=15), with total loss of NCAM-180 and PSA in the SCN had a shorter endogenous free-running period (τ; 23.5±0.09 h) and a longer activity time (α; 18.2±0.42 h) under constant darkness (DD) than wildtypes (n=12; τ=23.9±0.05 h [$P<0.05$]; α=15.8±0.87 h [$P<0.05$]). By 2 wk of DD the majority of mutant mice exhibited a desynchronous activity rhythm. Also, the photoentrainment of locomotor activity in NCAM-180 deficient mice which was unimpaired under the initial 12L:12D photoperiod (LD), was (in contrast to the wildtypes) disrupted after return to LD following DD exposure. Collectively, these findings are evidence that PSA-NCAM, and possibly NCAM-180, are important to the generation of circadian rhythms and may help maintain the coherence of these rhythms.

658.4

CHARACTERIZATION OF THE CIRCADIAN SYSTEM OF NGFI-A AND NGFI-A/NGFI-B KNOCKOUT MICE. T.S. Kilduff* (1), C. Vugrinic (1), S.L. Lee (2), J.D. Milbrandt (2), J.D. Mikkelsen (3), B.F. O'Hara (1), and H.C. Heller(1). (1) Center for Sleep & Circadian Neurobiology, Depts. of Psychiatry and Biological Sciences, Stanford Univ., Stanford, CA 94305, (2) Dept. of Pathology, Washington Univ., St. Louis, MO 63110, (3) Inst. of Med. Anatomy, Univ. of Copenhagen, Denmark.

The genes NGFI-A and NGFI-B have previously been shown to be induced in the suprachiasmatic nuclei by photic stimulation during the subjective night. The purpose of this study was to characterize the circadian system of mice in which either NGFI-A or both NGFI-A and NGFI-B were eliminated by homologous recombination. Wheel running activity was recorded from null mutants and wild type controls under LD 12:12 , DD, and LL conditions. All mice appear to entrain normally to a 12:12 cycle and could re-entrain to both phase advances and phase delays of the light cycle. The response of the pacemaker to acute light pulses is being assessed at the present time. These results, along with neuroanatomical information about the structure of the SCN in NGFI-A minus and NGFI-A/B minus mice, will be presented. (Supported by NIH grant AG11084).

658.5

IDENTIFICATION OF PINEAL-SPECIFIC GENES IN RAT BY DIFFERENTIAL DISPLAY PCR.

X. Wang* Michael J. Brownstein, and W. S. Young, III. Lab. Cell Biology, NIMH, NIH, Bethesda, MD 20892

In the mammalian pineal, the circadian production of melatonin is controlled by the rhythmic expression of acetyl-CoA: serotonin N-acetyltransferase (SNAT). Acetylation of serotonin by SNAT is the rate-limiting step in melatonin synthesis. Isoproterenol injections in intact rats stimulate the expression of SNAT or SNAT regulator(s) transcriptionally or translationally. Various cloning methodologies have failed to find the SNAT cDNA. In order to screen for pineal-specific mRNAs that may be crucial for the circadian regulation of melatonin synthesis, differential display PCR was employed. Pineal, retinal and cerebellar tissues were dissected from Sprague-Dawley rats (male, 200-250 each, Taconic Farms) two and half hours after intraperitoneal injections of 10 mg/kg isoproterenol. The same tissues were collected from uninjected rats as well. $T_{12}N_2$ oligos were used for cDNA synthesis using total RNA isolated from above tissue samples as templates. The same $T_{12}N_2$ oligos plus arbitrary 10-mers of random sequence were used to amplify the cDNAs by PCR in the presence of ^{35}S-dATP. The PCR products were displayed on a 6 % polyacrylamide gel. Clone PG10.2 is expressed in both the pineal gland and retina; the 8 kb mRNA is much more abundant in retina where it is expressed in the outer nuclear layer (especially at the outer edge) and over the photoreceptor cell bodies. *In situ* hybridization revealed that the clones PG23 and PG25 are pineal specific. Preliminary sequence analysis of cloned fragments shows no significant homologies to known genes. Full length cDNAs are being isolated from an isoproterenol-induced pineal cDNA library to see if they code for proteins with roles in the circadian regulation of melatonin synthesis.

658.6

CLONING OF THE MOUSE MEL$_{1a}$-MELATONIN RECEPTOR GENE. Alfred L. Roca* and Steven M. Reppert, Laboratory of Developmental Chronobiology, Mass. General Hospital, and Program in Neuroscience, Harvard Medical School, Boston MA 02114

Recently a high-affinity melatonin receptor, designated the Mel$_{1a}$-melatonin receptor, was cloned from mammals (Reppert et al. 1994; Neuron 13:1177). Defining the structure of the murine receptor gene is a necessary step for generating transgenic animals. Degenerate primers were designed using regions conserved among other mammalian Mel$_{1a}$-receptor cDNAs. PCR of mouse genomic DNA yielded a 466 bp fragment that was 94% identical at the amino acid level to the rat and Djungarian hamster Mel$_{1a}$-receptor cDNAs. In situ hybridization of adult C57BL/6J mouse brain using the PCR-generated fragment produced a hybridization pattern consistent with that expected for the Mel$_{1a}$-melatonin receptor; hybridization signal was most intense in the hypophyseal pars tuberalis. Southern blot analysis of genomic DNA indicated a single-copy gene. RNA was isolated from a murine cell line (RT2-2) which expresses the Mel$_{1a}$-receptor. Northern analysis of poly(A)+ RNA indicated a transcript length of ca. 1.9 kb. RT-PCR was used to generate the full-length coding region (1059 bp) of the receptor, which showed 84% amino acid identity to the human Mel$_{1a}$-receptor. RNase protection analysis, 5' and 3' RACE, and screening of a BALB/c mouse EMBL3 SP6/T7 genomic library revealed that the receptor gene consists of 2 exons divided by a large (>8 kb) intron. The 3' untranslated region is 444 bp long, and includes the polyadenylation signal AUUAAA. RNase protection assays suggest that a major transcription start site is located ca. 100 bp upstream of the initiation codon.

658.7

MELATONIN ACTION VIA PROTEIN KINASE C IN THE SCN OF THE RAT. A.E. Hunt, A.J. McArthur, M.U. Gillette*. Neuroscience Program and Depts. of Physiology and Cellular and Structural Biology. University of Illinois at Urbana-Champaign, Urbana, IL 61801.

Production of the pineal hormone melatonin occurs only at night, or during the dark phase of the subjective lighting cycle, and is controlled via a multisynaptic pathway from the suprachiasmatic nucleus (SCN) to the pineal gland. There are high-affinity melatonin receptors within the SCN itself, suggesting a feedback function for melatonin. Indeed, melatonin (10-9 M), when bath applied for 1 hr to a hypothalamic brain slice preparation, has been shown to advance the phase of circadian rhythm extracellular firing rate in the ensemble of SCN neurons. The SCN shows temporal sensitivity in that melatonin application during mid-day or mid-night is without effect, while application at subjective dusk (CT 10) or subjective dawn (CT 23) causes phase advances of about 4 hr. To study the signalling mechanisms responsible for melatonin's effects, the phorbol ester 12-O-tetradecanoyl phorbol 13-acetate (TPA), a potent protein kinase C activator, was bath applied to brain slices (10-8 M, 10 min) and was found to reset the SCN firing rate with a profile similar to that of melatonin. The specific protein kinase C inhibitor, Calphostin C, which binds and blocks the diacylglycerol site on PKC, blocks melatonin phase shifts when bath applied 45 min prior to melatonin treatment at CT 10 and CT 23. Calphostin C induces no phase shift of its own when applied alone. Our data indicate that melatonin can phase shift circadian timing within two windows of sensitivity and that melatonin exerts its effects at both dusk and dawn via a protein kinase C pathway. (Supported by PHS grant NS22155).

658.8

PONTINE CHOLINERGIC NEURONS SHOW FOS-LI IN ASSOCIATION WITH CHOLINERGICALLY-INDUCED REM SLEEP. P. Shiromani*, S. Winston, R.W. McCarley. *Brockton VA-Harvard Med School, MA 02401.*

We (Brain Res, 1992; J Neurosci,1995) and others (Yamuy et al., J Neurosci, 1993) have shown that Fos-LI occurs in pontine areas in response to cholinergically-induced REM sleep (REMc). Since pontine cholinergic mechanisms have been implicated in REM sleep generation, in the present study we examined whether pontine cholinergic neurons show Fos-LI in association with REMc.

Sleep was recorded from cats given pontine microinjections of either saline (n=2; 0.25μl) or carbachol (n=3; 2 ug/0.25μl). Carbachol treated animals had REMc (27-40 min) and were sacrificed (nembutal) at end of REMc. In saline treated animals, very few Fos-LI cells were found in the pons. None of these cells were colocalized in LDT-PPT cholinergic neurons. On the other hand, the animals with REMc showed a consistent pattern of Fos-LI cells in the dorsolateral pons. In the REMc animals, a subpopulation of the LDT-PPT cholinergic cells were also Fos-LI positive (contralateral= 10.17%; ipsilateral=11.1%).

We found that only a subpopulation (10.64%) of LDT-PPT cholinergic neurons were also Fos-LI positive. It is interesting that single-unit studies have also found that only a subpopulation of neurons recorded in the LDT-PPT are "REM-on". Thus, even in a phenotypically similar population (i.e., cholinergic) only a subset are differentially affected during REM sleep, as assessed by electrophysiological or molecular techniques.

658.9

CHANGES IN c-FOS, PPE AND DYN mRNA LEVELS ASSOCIATED WITH THE PLATFORM TECHNIQUE OF REM SLEEP DEPRIVATION. L.Ramanathan*, R.Basheer, R.W. McCarley and P.Shiromani. *VA-Harvard Medical School, West Roxbury, MA 02132.*

Immobilization stress, cold water swim, and chronic or acute drug treatments alter c-fos, preproenkephalin (PPE) and dynorphin (DYN) mRNA levels. c-Fos is known to induce the expression of both PPE and DYN. In the present study, changes in the expression of these messages were investigated using the platform technique of REM sleep deprivation. Rats were placed on either large platforms (LP) or small platforms (SP) for 1 and 5 days, while control animals were kept in dry cages throughout the experimental period.

Northern blot analysis showed decreased levels of c-fos mRNA in the cortex and striatum of platform animals compared to rats maintained in dry cages. In contrast, PPE mRNA levels were increased in the cortex and striatum of platform animals. DYN mRNA levels were unaltered.

The differential expression of messages whose protein products have been associated with various aspects of the transcriptional cascade described in other systems, suggests that a similar series of events may play a role in this experimental paradigm.

658.10

CHANGES IN c-FOS AND ChAT mRNA LEVELS IN SPECIFIC RAT BRAIN REGIONS DURING NORMAL SLEEP. M. A. Greco[1], G. Perides[2*], R.W. McCarley[1], and P. Shiromani[1]. Harvard Medical School, VA Medical Center, West Roxbury, MA 02132, and New England Medical Center, Boston, MA 02111

Studies in this laboratory have investigated neuronal mechanisms underlying sleep-wake states. These studies have also shown an increased number of c-fos immunoreactive cells in a subpopulation of pontine cholinergic neurons in response to drug-induced REM sleep. We have new begun to examine molecular events associated with physiological sleep. Initial experiments using northern blot analysis indicated a more sensitive assay was required to investigate possible transcriptional regulation mechanisms of gene expression in this system. In the studies described here, c-fos and ChAt mRNA levels from discreet brain regions were analyzed across normal sleep states. Rats were sacrificed during waking, slow wave sleep or REM sleep. Total RNA was extracted from punch biopsies of the medial PRF, DRN, LC and LDT regions of the pons as well as hypothalamic areas. Messenger RNA levels were analyzed by quantitative RT/PCR. The results indicate that c-fos and ChAT messages are differentially and variably expressed. These studies show the utility of RT/PCR to analyze low abundance messages in specific areas of the brain.

658.11

CHANGES IN TYROSINE HYDROXYLASE mRNA LEVELS ASSOCIATED WITH THE PLATFORM TECHNIQUE OF REM SLEEP DEPRIVATION R.Basheer*,R.W.McCarley and P.Shiromani. *Dept Psychiatry, Harvard Medical School- VA Medical Center, West Roxbury, MA 02132.*

A number of experimental techniques have been shown to induce the expression of tyrosine hydroxylase mRNA in locus coeruleus. We studied alterations in TH mRNA using a paradigm that also produces REM sleep deprivation in rats.

Rats were placed on large platform (LP) and small platform (SP) for one and five days and compared with controls kept in dry cages. Changes in TH mRNA were analysed by northern analysis of total RNA isolated from pons, locus coeruleus and ventral tegmental area. Preliminary results showed that compared to dry cage controls there was no significant change in TH mRNA in LP rats. Whereas in SP animals there was a significant increase in TH mRNA after one day which drops to control levels after five days. It will be important to examine the changes in TH mRNA in discrete pontine nuclei using *in situ* hybridization.

These data indicate that TH mRNA levels are changed with the pedestal treatment.

658.12

ULTRAVIOLET-SENSITIVE RETINAL PHOTORECEPTORS MEDIATE LIGHT-INDUCED PHASE-SHIFTS AND FOS EXPRESSION IN RAT SUPRACHIASMATIC NUCLEUS. S. Amir* and B. Robinson. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada.

Mammalian circadian rhythms are generated by a pacemaker located in the hypothalamic suprachiasmatic nucleus (SCN) and are entrained by photic stimuli that are carried to the SCN in a direct retinal projection, the retinohypothalamic tract (RHT), and in an indirect projection from the intergeniculate leaflet (IGL), the geniculohypothalamic tract (GHT). The retinal photoreceptors underlying entrainment, and that are thought to be distinct from those involved in vision, have not been identified. In rodents, photic stimuli that entrain circadian rhythms induce expression of the transcription factor Fos in SCN cells innervated by the RHT and GHT. Here we report that in rats ultraviolet light pulses known to excite the retina electrophysiologically induce a pattern of Fos expression in the SCN that corresponds to the known pattern of distribution of RHT and GHT terminals in the SCN region. The effect of ultraviolet light on Fos expression is phase dependent and appears to be mediated by a retinal mechanism bearing characteristics of a specific ultraviolet receptor. Because we find in addition that ultraviolet light pulses that induce Fos expression in the SCN are sufficient to induce phase shifts in circadian rhythms, we conclude that the retinal photoreceptors that mediate the effect of light on rodent circadian pacemaker are ultraviolet sensitive.

658.13

DAY-NIGHT DIFFERENCE IN THE NUMBER OF FOS-IMMUNOREACTIVE NEURONS IN THE SUPRACHIASMATIC NUCLEUS OF FETAL SHEEP.
S.Breen[1], S.Rees[1], L.Wise[3]* & D.Walker[2]. [1]Department of Anatomy & Cell Biology, University of Melbourne, Parkville, Victoria 3052, Australia; [2]Department of Physiology & [3]Department of Psychology, Monash University, Clayton, Victoria 3168, Australia.

Fetal sheep exhibit several behavioural and hormonal diurnal rhythms in late gestation. It is unclear whether these rhythms are controlled directly by the fetal suprachiasmatic nucleus (SCN) or result from diurnal changes in maternal hormones. FOS immunoreactivity (FOS-ir) was utilised as a marker of neuronal activation to determine if SCN neurons are active during gestation. Pregnant ewes (n=28) were maintained under a 12 hour (h) light-dark cycle prior to administration of an overdose of sodium pentobarbitone at either 1200 h or 0300 h. At 1200 h in the 75 d fetus (term=146 d) a few FOS-ir neurons were observed while in the 90, 105, 117 and 138 d fetuses many FOS-ir neurons were present throughout the nucleus. However fetuses killed at 0300 h at 90 and 138 d gestation showed only a few FOS-ir neurons in the SCN. To determine whether the activity of the fetal SCN was due to direct light stimulation of the fetal retina through the maternal body wall, 1 fetus from each of 2 sets of twins was optically enucleated a 100 d. The unoperated twin acted as a control. Counts of FOS-ir neurons in the SCN of these animals at 1200h at 133 d showed no difference between operated and control animals, indicating that retinal input is not essential for entraining the fetal SCN. Another possibility is that a chemical messenger which undergoes diurnal fluctuation in the mother may communicate time of day information to the fetus. Two ewes each with twin fetuses of 133 d were exposed to constant light conditions for 5 days. Analysis of these fetuses indicated that the day-night difference in FOS-ir neuron number had now been abolished, suggesting that a messenger such as melatonin may be important in entraining the fetal SCN.

658.14

LIGHT-INDUCED AND CIRCADIAN EXPRESSION OF *fosB, c-fos, junB* AND *c-jun* IN THE HAMSTER SUPRACHIASMATIC NUCLEUS.
M. E. Guido*, B. Rusak and H. A. Robertson. Departments of Psychology and Pharmacology, Dalhousie University, Halifax NS, Canada B3H 4J1.

The mammalian suprachiasmatic nucleus (SCN) of the anterior hypothalamus is the site of an endogenous pacemaker that is responsible for the timing and overt expression of daily rhythms. The circadian rhythmicity of this pacemaker is synchronized by light, and a molecular correlate of photic entrainment is the rapid and transient induction of various immediate early genes (IEGs) in SCN cells. These IEGs encode for, among other things, transcription regulatory factors such as Fos and Jun family proteins. The anatomical pattern of cFos induced by light is similar to the pattern of retinohypothalamic fiber terminals in the SCN. Fos and Jun proteins could be regulating transcriptionally the expression of genes responsible for the timing of the intracellular circadian pacemaker.

To study the role that other IEGs could be playing in the SCN, we have characterized the photic and circadian expression of *fosB* as well as *c-fos, c-jun* and *junB* by *in situ* hybridization in hamsters. *fosB* mRNA exhibits a typical IEG kinetic of induction in response to a pulse (30 min) of light (during the dark phase of an LD 14:10 schedule) in cells of SCN, and mRNA levels are elevated for up to 150 min after the light pulse. When we assessed the spontaneous and light-evoked expression of these genes at different circadian phases, we found light-induced expression for all these genes during the subjective night, but *c-fos,* and especially *junB* also showed increased expression either spontaneously or after a light pulse at the beginning of the subjective day. These results suggest that induction of some IEGs in the SCN occurs spontaneously as well as in response to light stimuli at selected circadian phases.
(Supported by CONICET of Argentina and the MRC of Canada).

658.15

FOS IMMUNOREACTIVITY IN THE SUPRACHIASMATIC NUCLEUS (SCN) OF THE DIURNAL RODENT ARVICANTHIS NILOTICUS.
Catherine Katona, Cheryl Sisk* & Laura Smale. Department of Psychology/Neuroscience Program, Michigan State University, East Lansing, MI 48824

The suprachiasmatic nucleus (SCN) regulates circadian rhythms in all mammals examined to date and has been studied extensively in nocturnal species. Little is currently known about differences in the neural mechanisms controlling rhythms in nocturnal and diurnal animals. These functional differences may exist either within the SCN or in response to SCN signals. We used Fos as an index of SCN function to explore the hypothesis that mechanisms within the SCN are coupled in one way to the LD cyle in nocturnal animals and are coupled oppositely in diurnal animals.

Fos is a phosphoprotein which interacts with DNA to regulate gene transcription. The number of Fos immunoreactive (Fos-IR) cells in the SCN of a nocturnal rodent, the rat, peaks during the light phase of a 12:12 light-dark (LD) cycle. We examined Fos-IR in the SCN of a diurnal rodent, Arvicanthis niloticus.

Our study included 36 adult males sacrificed at 6 time points around a 12:12 LD cycle. There was the suggestion of a 24h rhythm of Fos-IR within the SCN of A.niloticus. During the light part of the cycle there was an increase in the number of cells expressing Fos-IR, whereas during the night, levels of Fos-IR were lower.

Thus, the rhythm of Fos-IR in the SCN is similar in the nocturnal laboratory rat and the diurnal species A.niloticus in a 12:12 LD cycle; however, it remains possible that temporal differences of Fos-IR occur in different subsets of SCN cells.

658.16

MICROINJECTION OF DOUBLE-STRANDED DNA OLIGONUCLEOTIDE CONTAINING AP-1 SEQUENCE BLOCKS LIGHT-INDUCED PHASE ADVANCE OF HAMSTER CIRCADIAN RHYTHMS
J. Takeuchi, D. Golombek and M. Ralph* Dept.of Psychology, University of Toronto , Toronto, ON, Canada M5S 1A1

Expression levels of a variety of AP-1 transcription factor proteins including C-Fos, Fos B, C-Jun, Jun B and Jun D in the suprachiasmatic nucleus (SCN) are reported to change in response to a phase-shifting light pulse . Although a role for these proteins in the phase shift mechanisms is suggested, few data are available to directly prove their involvement. A clear interpretation of results from a gene knock-out (c-fos) remains difficult partly because of the diversity of these protein complexes. One way to assess the direct involvement of these factors is to reduce their bindings to AP-1 DNA sequences in the SCN *in vivo* by competitively adding double-stranded(ds) DNA oligonucleotide containing AP-1 sequences. After implantation with a guide cannula aimed at the bottom of the third ventricle near the SCN, hamsters were housed in constant darkness and their wheel running activities were monitored. One ul of 36-mer ds AP-1 oligo (50 uM or picomoles/ul) or control oligo with shuffled AP-1 sequence in artificial cerebrospinal fluid was injected through the cannula under dim safe-light conditions 10-15 minutes before light exposure at circadian time 18 (250-350 lux for 15 minutes). The animals remained active after injections. AP-1 oligo pretreatment significantly reduced light-induced phase advances (13.2±13.5 min. n=10) while control oligo pretreatment did not (93.9±47.8 min. n=8).

These results suggest that binding of the AP-1 transcriptional complexes to AP-1 sequences is an essential part of light-induced phase advances in the mammalian circadian clock.

658.17

CIRCADIAN REGULATION OF CREB PHOSPHORYLATION IN THE SUPRACHIASMATIC NUCLEUS BY LIGHT, GLUTAMATE, AND NITRIC OXIDE.
J. M. Ding*, W. J. Hurst, L. E. Faiman, L. R. Kuriashkina, and M. U. Gillette. Dept. of Cell & Structural Biology, Physiology, and Neuroscience Program. University of Illinois, Urbana, IL 61801.

Circadian rhythms of mammals are timed by an endogenous clock with a period of about 24 hours located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Light synchronizes this clock to the external environment by daily adjustment in the phase of the circadian oscillation. The SCN receives direct visual input via the retino-hypothalamic tract (RHT) which is thought to release the excitatory neurotransmitter glutamate (Glu). We tested the hypothesis that light adjusts the SCN clock through activation of N-methyl-D-aspartate (NMDA) receptor, and subsequently induces calcium dependent activation of nitric oxide synthase (NOS) in the SCN resulting the release of nitric oxide (NO). Brief exposure of rats to light only at the night-phase of the circadian cycle, induces phosphorylation of the transcription factor cAMP response element binding protein (CREB) in the SCN on the transcriptional regulatory site (Ser 133). Brief treatment of SCN *in vitro* with Glu produced light-like phase shifts and phosphorylation of CREB. Furthermore, antagonists of NMDA or NOS blocked Glu induced phase shifts as well as CREB phosphorylation. Moreover, DNA mobility assay of SCN tissue demonstrated CRE binding ability of phospho-CREB after light and Glu treatment at night. These results suggest the following signal transduction pathway linking environmental light stimulus to molecular changes in the SCN that reset the phase of the biological clock: Glu release--NMDA receptor activation--NOS stimulation--NO production--CREB phosphorylation--target gene activation--phase shift.
(Supported by NIH (NS22155 and NS33240) and AFOSR (F-49620-93-1-043).

658.18

CYCLIC-GMP-DEPENDENT PROTEIN KINASE INHIBITORS BLOCK LIGHT-INDUCED PHASE ADVANCES OF CIRCADIAN RHYTHMS IN VIVO.
A. Mathur *, D. A. Golombek and M. R. Ralph. Dept. of Psychology, University of Toronto, Canada, M5S 1A1.

Biological rhythms in nature and in the laboratory can be synchronized by 24 hr. cycles of light and dark. Synchronization is thought to be accomplished primarily through daily phase delays and advances of the endogenous circadian rhythm which in mammals is generated by the hypothalamic suprachiasmatic nucleus (SCN). Pulses of light are known to shift the phase of activity rhythms of nocturnal animals but only during the time of their cycle (Circadian Time or CT) when they are active (subjective night). In the SCN, a number of second messenger pathways may participate in photic signal transduction. *In vitro,* pulses of both c-AMP and c-GMP analogs induce CT-dependent phase advances of electrical activity in SCN slices (Prosser, McArthur and Gillette, 1989). The involvement of cyclic nucleotide -dependent protein kinases was examined *in vivo* using inhibitors of c-AMP-dependent kinase (PKA) and c-GMP-dependent kinase (PKG) microinjected near the SCN of hamsters. In a constant dark, aperiodic environment, selective (KT-5823, 20 μM) and non-selective (H-8, 20 μM) inhibitors of PKG had no effect on phase delays of wheel-running activity rhythms produced by 15 min. light pulses given in the early subjective night, but significantly attenuated (70 %) phase advances induced late in the subjective night (CT 18). A selective inhibitor of PKA (H-89, 1 μM) had no effect at either time point. The results suggest that PKG activity is necessary but not sufficient for normal photic responsiveness, and that PKA is not required. The phase dependence of the effect of PKG supports the notion that photic responsiveness at different phases of the circadian cycle are mediated by different biochemical pathways. (Supported by the AFOSR grant to M. R. R.)

658.19

CALMODULIN-DEPENDENT PROTEIN KINASE II EXPRESSION DURING WAKEFULNESS AND SLEEP. M.Pompeiano*, P.Arrighi, C.Cirelli and G.Tononi. Inst. Biol. Chem. and Dept. Physiol. Biochem., Univ. of Pisa, Italy, I-56100.

There is strong evidence that wakefulness (W) and sleep (S) are associated with changes in the expression of certain genes, including transcription factors, in specific brain areas. Among potential target genes are those coding for the subunits of calmodulin-dependent protein kinase II (CaM-K II), which has been implicated in synaptic plasticity. We examined the expression of CaM-KII mRNA in the brain of rats sacrificed after spontaneous W or S and after sleep deprivation (SD).

Two groups of rats were killed at 2PM either after a long period of S(n=6) or at least 30min of W(n=6). A third group of rats was sacrificed at 2AM after a long period of spontaneous W(n=6). 3 animals from each group were used for in situ hybridization (ISH) and 3 for Northern blot (NB) analysis. Rats underwent manual SD for 3h(n=6), 6h(n=4), 12h(n=5), and 24h(n=6) starting at 8AM, and for 12h(n=6) starting at 8PM. Controls were sacrificed at the same phase of the 24h cycle.

The expression of CaM-K II is strongly and differentially modulated during spontaneous S-W states, as shown with both ISH and NB: the α subunit mRNA increases during spontaneous W in the cerebral cortex and hippocampal formation, while in the same areas CaM-K II ß mRNA decreases during W with respect to S. After SD, the changes in the expression of both subunits of CaMK-II in the cerebral cortex and hippocampus were less marked than after spontaneous W.

Given the involvement of CaM-K II in long-term potentiation, kindling and learning, the observation of changes in its expression in specific brain areas during spontaneous and forced W suggests potential differences in synaptic functioning during different arousal states.

658.20

LONG-TERM IMAGING OF INTRACELLULAR CALCIUM IN CELLS OF THE SUPRACHIASMATIC NUCLEUS. Michael E. Geusz* and Gene D. Block. NSF Center for Biological Timing, Department of Biology, Univ. of Virginia, Gilmer Hall, Charlottesville, VA 22903.

The use of Ca^{2+}-sensitive fluorescent dyes such as Fura-2 to measure intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) is often limited by time-dependent loss of dye from the cell and its gradual compartmentalization into regions that are not representative of cytosolic Ca^{2+}. Dextran-conjugates of Fura-2 circumvent these restrictions but require microinjection, limiting their usefulness with small neurons such as those of the suprachiasmatic nucleus (SCN). Because the SCN is the principal mammalian circadian pacemaker we wished to monitor $[Ca^{2+}]_i$ in SCN cells for at least 24 hours. Such a capability would provide $[Ca^{2+}]_i$ measurements in individual SCN cells throughout the circadian cycle and would allow the effects of phase-shifting agents on $[Ca^{2+}]_i$ to be measured at different circadian phases.

For long-term Ca^{2+} imaging, primary cell cultures of neonatal rat SCN were loaded with the dye Fura-PE3 AM, a modified form of the membrane-permeable Fura-2 AM which is reported to show less compartmentalization and leakage than Fura-2. Cells were loaded for 1 to 2 hours with 10 μg/ml Fura-PE3 AM and then left at room temperature (22°C) in a HEPES-buffered medium. The emission at 510 nm was imaged during alternating 340 and 380 nm light pulses. The cells showed a diffuse cytoplasmic fluorescence for at least 50 hours after loading and elevated K^+ treatments caused repeatable $[Ca^{2+}]_i$ increases in neurons and astrocytes during this time. This approach can be used to measure $[Ca^{2+}]_i$ in SCN cells during the circadian cycle. *Supported by NIH NS15264 and the NSF Center for Biological Timing.*

658.21

TTX AND Mg^{2+} RESISTANT SLOW FLUCTUATIONS OF THE MEMBRANE POTENTIAL IN SUPRACHIASMATIC NUCLEI NEURONS *IN VITRO*. L. Trachsel*, K. Kampe, H.U. Dodt, and W. Zieglgänsberger, Max Planck Institute of Psychiatry, Clinical Institute, Clinical Neuropharmacology, 80804 Munich, FRG.

We used spontaneous fluctuations of the membrane potential of SCN neurons as an integral measure to assess neuron ensemble activity as a function of synaptic mechanisms and circadian control. Neurons in cell clusters in rat SCN slices (300 μm; at 35-37 °C, pH 7.4) were visually identified by IR videomicroscopy[a], and voltage clamped at -50mV. Power spectra (0.016-1000 Hz) of the holding current (r.m.s. ~50 pA) were decomposed into Lorentzian components[b]. Oscillations below 10 Hz prevailed in terms of spectral power in both the day and night SCN, and with TTX and Mg^{2+} perfusion. The spectral distributions were fitted (r^2>0.9) with Lorentzian functions. In the SCN corresponding to subjective day, the corner frequencies (f_0) of two Lorentzian components were 9 Hz and 180 Hz. In the nighttime SCN, the f_0 were estimated at 11 Hz and 22 Hz. Power between 1-3 Hz was significantly lower, and power between 70-630 Hz was significantly higher in neurons of the day SCN than of the nighttime SCN ($p<0.05$). TTX significantly suppressed power between 50-500 Hz. Only one Lorentzian component was found (f_0 at 10 Hz) in both circadian preparations under TTX. High Mg^{2+} and zero Ca^{2+} perfusion evoked similar effects. We conclude that SCN neurons held below spiking threshold operate electrophysiologically in a wide frequency range of 1 ms - 64 s periodicity, where slow frequencies of <10 Hz clearly predominate. The TTX and Mg^{2+} sensitive high frequency Lorentzian (200 Hz) suggests fast synaptic sources. The TTX and Mg^{2+} resistant low frequency Lorentzian (<10 Hz), suggests either non-synaptic input, or intrinsic slow rhythm generators in SCN neurons.

[a]Dodt HU, and Zieglgänsberger W (1994) Infrared videomicroscopy: a new look at neuronal structure and function. *Trends Neurosci* 17: 453-458. [b]DeFelice LJ. *Introduction to Membrane Noise*. Plenum Press, New York, 1981.

658.22

CONSTANT LIGHT HOUSING INDUCES FOS PROTEIN IN RAT INTERGENICULATE LEAFLET. K. Edelstein* and S. Amir. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada H3G 1M8.

Exposure to prolonged constant light (LL) disrupts circadian rhythms in nocturnal rodents. This disruption may be manifested as a loss of circadian rhythmicity, desynchrony of individual rhythms, or splitting of circadian activity rhythms into two or more components. The neural mechanisms underlying these effects are not well defined, although previous research has demonstrated that ablation of the intergeniculate leaflet (IGL) prevents splitting of circadian activity rhythms in the hamster. We examined the effects of LL on the circadian system of Wistar rats previously housed under a normal light-dark cycle, using a telemetry system to measure temperature and activity rhythms, and the immunocytochemical expression of Fos protein as a marker of light-induced activation of cells in the IGL. Results indicate that LL induces Fos expression in the IGL independent of circadian time. Furthermore, we found a correlation between the disruption of circadian temperature and activity rhythms observed in rats housed in LL for 30 days, and attenuation of Fos immunoreactivity in the IGL of those animals. Whereas Fos expression was observed in the IGL of rats sacrificed during the LL period prior to the loss of circadian rhythmicity, rats sacrificed after this disruption occurred tended to exhibit fewer Fos immunoreactive cells in this region. Results support the idea that the IGL mediates the disruption of circadian rhythms during exposure to prolonged LL. These data also suggest that Fos expression in the IGL may be a marker of the integrity of the circadian system in rats housed under prolonged LL.

658.23

Fluorocitrate Affects Circadian Rhythms in Rats. A.S. Elliott* and A.A. Nunez. Dept. of Psych., Neuroscience Program, Michigan State University, East Lansing, MI 48824.

The glial metabolic inhibitor fluorocitrate was used to probe the role of astrocytes in the generation and expression of circadian running wheel and drinking rhythms. Rats (≈180 g) received implants of double cannulae such that the tips of the cannulae were aimed to end immediately dorsal to the suprachiasmatic nucleus (SCN). Animals were placed in running wheels with food and water available ad lib. under dim red illumination. After rhythms were established, animals received an injection of 2 nmol fluorocitrate in each cannula at either the beginning or end of their active phase, and rhythms were subsequently monitored. Wheel running and drinking were subdued for about 24 hr and, upon resumption, showed various changes from previous patterns. A large proportion of animals showed a phase advance regardless of the time of injection. A subset of the animals showed no phase shift but a lengthening of tau, and a small proportion of the animals became "arrhythmic". This arrhythmic pattern persisted even when the animals were placed under a light:dark cycle. Lower doses (1nmol fluorocitrate / cannula) consistently produced a lengthening of *tau*. Taken together, these results support the hypothesis that astrocytes in the SCN play an intimate role in the generation and maintenance of circadian rhythms.

658.24

CIRCADIAN CHANGE IN THE INTENSITY OF ULTRAWEAK BIOCHEMILUMINESCENCE FROM RAT SUPRACHIASMATIC NUCLEUS SLICE. Y. Isojima*[1,2], T. Isoshima[3], K. Nagai[1], K. Kikuchi[4] and H. Nakagawa[1]. [1]Division of Protein Metabolism, Institute for Protein Research, Osaka Univ., Suita, Osaka 565, Japan. [2]Photodynamics Research Center, The Institute of Physical and Chemical Research (RIKEN). [3]Frontier Reasearch Program, The Institute of Physical and Chemical Research (RIKEN). [4]Faculty of Engineering, Univ. of Tokyo.

Two classes of light emission related to biological phenomena are known. One is the well-known bioluminescence visible to human eyes which is associated with reactions such as the luciferin-luciferase system. The other is ultraweak biochemiluminescence in the order of 10^{-17}W/mm^2 or less, which is spontaneously emitted from all kinds of living organisms without excitation light. In the previous work, we developed ultrahigh sensitivity photodetection system using a silicon avalanche photodiode and succeeded to detect ultraweak biochemiluminescence from rat hippocampal slice. It was shown that the intensity of biochemiluminescence is at the order of 10^{-18}W/mm^2 and related with neural activity. In the present work, to study the oscillation mechanism of circadian clock in the suprachiasmatic nucleus (SCN), we applied this phtodetection system to the SCN slice culture and succeeded to detect ultraweak biochemiluminesence for as long as 72 hours. The intensity of biochemiluminescence showed circadian change, though its pattern was quite different from electrical or metabolic activity of the SCN. The intensity showed peaks in the middle of subjective night as well as subjective day, and troughs in the transition periods of the day and night. This result suggests that ultraweak biochemiluminescence from the SCN reflects not only neural activity but also other cellular activity.

658.25

VARIOUS TYPES OF SLEEP DEPRIVATION HAVE DIFFERENT EFFECTS ON CEREBRAL PROTEIN SYNTHESIS IN THE RAT. P. Ramm and R.K. Zoltoski, Dept. of Psychology, Brock University, St. Catherines, ON L2S 3A1, Canada

We investigate possible restorative functions of sleep by mapping cerebral protein synthesis (CPS) during sleep-wake states. In this study, we compared CPS during post-deprivation recovery sleep under three conditions: a) a short period of manual total sleep deprivation; 24 hr of island platform mixed sleep deprivation, and computer-controlled total sleep deprivation.

Rats received electrodes for recording of EEG and EMG, and cannulae allowing external access to the femoral artery and vein. A 3 day period was then allowed for recovery from surgery. Control animals (n=16) were manually prevented from entering sleep for 4-6 hr. This condition represents minimal deprivation and a uniform high level of sleep need. A 24 hr platform condition (n=11) used medium-sized islands over water. This condition replicates classic REM sleep deprivation. A final deprivation condition (n=9) used 24 hr in a computer-driven rocking cage, which was produced total sleep deprivation (> 99%). Following the deprivation period, the animals were allowed to sleep. L-1[14C]leucine was injected, timed plasma samples were taken for LSC and analyses of free amino acids, and the EEG/EMG were recorded for state monitoring. After 45 min, the brains were extracted, cut in a cryostat, and processed for quantitative autoradiographic analysis of CPS.

In the minimal deprivation (tired) and platform (some SWS deprivation, total REM deprivation) conditions, we failed to observe a relation between recovery SWS and rates of CPS. In contrast, the rocker deprivation group (total sleep deprivation) exhibited a strong ($p < 0.01$) negative relation between CPS and SWS. These data suggest that the metabolic consequences of classic REM sleep deprivation are different from those of total sleep deprivation.

658.26

Recovery sleep in Fos-LacZ mice leads to a rapid decline of the elevated Fos and ß-gal levels which result from forced waking. J.E. Sherin, P. Shiromani, J. Morgan and C.B. Saper, Harvard Med. School, Boston MA 02115 & Roche Inst. of Molecular Biology, Nutley N.J. 07110.

Previously we reported that sleep-deprivation (SD) increases the level of Fos-protein in the rat and cat brain, and that recovery sleep (RS) following SD leads to its rapid decline. Using transgenic Fos-LacZ mice (n=6), we examined the effects of SD and RS on Fos and ß-gal levels in brain. Mice were sleep-deprived for 3 hrs. Three mice were sacrificed for analysis immediately after deprivation while remaining animals (n=3) were allowed to sleep or wake spontaneously for 1.0,1.5 and 2.0 hours, at which point they were sacrificed for analysis.

Brains from animals not allowed recovery sleep contained high levels of ß-gal and Fos which were particularly prominent in cingulate and piriform cortices. In contrast, brains from animals which slept spontaneously following deprivation showed a dramatic decrease in ß-gal and Fos.

These preliminary results in Fos-LacZ mice corroborate findings in rat and cat that Fos and now ß-gal protein degradation is enhanced during sleep. We propose that an increase in degradative processes at the cellular level during sleep may be central to the functions of sleep. Thus, the efficient elimination of gene products that accumulate during waking may serve a restorative function at the cellular and ultimately the organismal level.

INVERTEBRATE LEARNING AND BEHAVIOR V

659.1

PHOSPHORYLATION OF THE CONDITIONING-ASSOCIATED GTP-BINDING PROTEIN CP20 BY PROTEIN KINASE C. T. J. Nelson*, C.-L. Yi and D. L. Alkon. Laboratory of Adaptive Systems, NINDS, National Institutes of Health, Bethesda, MD 20892.

The phosphorylation state of cp20, a low-MW membrane-associated GTP-binding protein, was previously shown to increase 2- to 3-fold in *Hermissenda* 24h after associative conditioning. Iontophoretic injection of either cp20 or protein kinase C (PKC) reproduces the biophysical and structural modifications that accompany memory acquisition. Associative conditioning causes translocation of PKC from the cytosol to membrane. Here, cp20 is shown to be phosphorylated by PKC. Marked differences in activity were observed with the three major isoforms of PKC, while casein kinase, CaM-kinase II, and cyclic AMP-dependent protein kinase produced no detectable phosphorylation of cp20, suggesting that the increase in phosphorylation of cp20 after conditioning is due to PKC. Phosphorylation of cp20 had no effect on its GTPase or GTP binding activity, but caused a translocation of cp20 from cytosol to the nuclei / mitochondrial fraction.

659.2

GABA-INDUCED FACILITATION AT TYPE B TO A PHOTORECEPTOR SYNAPSES IN *HERMISSENDA*. L.M. Schultz* and G.A. Clark. Program in Neuroscience, Psych. Dept., Princeton University, Princeton, NJ 08544.

Enhanced type B photoreceptor excitability is a well-documented neuronal correlate of associative learning in *Hermissenda*. However, recent evidence suggests that facilitation at type B to A cell synapses may also contribute to learning in this system. Serotonin (5-HT) has been implicated in both type B cell excitability and synaptic strength changes, and γ-aminobutyric acid (GABA) has more recently been linked to type B cell excitability changes. Here we examined whether GABA would also promote facilitation at type B to A cell synapses.

Rapid bath perfusion with 100 μM GABA followed by a seawater rinse (GABA + RINSE) enhanced the amplitude of inhibitory postsynaptic potentials (IPSPs) elicited in type A cells by type B cell stimulation (mean change ± SEM: +0.99 ± 0.18 mV; $t_9 = 5.56$, $p < .0005$), as did 10 μM 5-HT (+0.53 ± 0.18 mV; $t_9 = 2.92$, $p < .01$), in confirmation of previous results. By contrast, preparations continually perfused with either seawater (-0.33 ± 0.18 mV; $t_9 = 1.85$, NS) or 100 μM GABA (-0.24 ± 0.21 mV; $t_9 = 1.13$, NS) showed no significant changes. An ANOVA ($F_{3,36} = 11.86$, $p < .0001$) followed by Duncan pairwise comparisons revealed that the changes in IPSP amplitude observed in the GABA + RINSE and 5-HT conditions were both significantly greater than the changes in the other two conditions (p's < .01), which did not differ. The observation that GABA + RINSE, but not sustained GABA exposure, promotes synaptic facilitation suggests that when GABA is present, its slow, modulatory effects on synaptic strength may be counteracted by its faster shunting actions; hence, removal of GABA from the bathing medium may have revealed its more sustained modulatory actions. Because the GABA + RINSE condition approximates the transient US-mediated activation of GABAergic hair cells which occurs during associative training of the intact animal, these findings suggest a straightforward means by which behavioral training could promote synaptic facilitation in this system.

659.3

VOLTAGE-SENSITIVE DYE RECORDING FROM THE *APLYSIA* ABDOMINAL GANGLION INDICATES MODEST LE CELL RESPONSE TO A LIGHT SIPHON TOUCH. C. Hickie*, L.B. Cohen, and P.M. Balaban. Dept. of Physiol., Yale Univ. Sch. of Med., New Haven, CT 06520, and Inst. of Higher Nervous Activity and Neurophysiol., Moscow.

Recent reports suggest that the LE sensory neurons may not be activated by a light touch that initiates a gill-withdrawal reflex. We have used voltage-sensitive dye recordings to evaluate the role of LE sensory neurons in the generation of gill withdrawals. In recordings made with the abdominal ganglion in sea water, we detected action potentials in about 100 neurons in response to a 1 or 2 g touch. The gill withdrawal in response to this stimulus was vigorous and often seemed to be more than half as large as the response to a 5 g touch. Additional recordings were made in a low calcium or a high divalent sea water. This suppressed the activity of interneurons and motor neurons but presumably did not affect the response of sensory neurons. In experiments on five animals we estimated the number of cells meeting LE-cell criteria (i.e. small, no spontaneous activity, activated by touch in altered divalency sea water, located in the LE region). In response to a light touch a median of 5 cells (range 0 to 8) fired a median of 1.6 spikes/cell (range 0 to 2.9). Given the small PSP size in gill motor neurons from LE spikes relative to the overall PSP size in responses to siphon touch (Byrne et al., *J.Neurophysiol*, 1978), this low activity level is consistent with the existence of other sensory cells responsive to light touch. We estimate that the LE contribution to the motorneuron PSP for light to moderate touches ranges from 0 to 10% depending on the strength of the touch and the behavioral state of the preparation. We are presently recording from other regions of the abdominal ganglion and from other ganglia to search for cells briskly activated by siphon touch in altered divalency sea water. Supported by NSF grant IBN-9222214, and NIH grants NS08437 and NS07102.

659.4

NOCICEPTIVE RESPONSES AND SENSITIZATION OF LE SIPHON SENSORY NEURONS IN *APLYSIA*. P.A. Illich* and E.T. Walters. Dept. of Integrative Biology, Univ. Texas - Houston Medical School, TX 77030.

Responses of LE sensory cells to mechanical stimulation were compared in 1) a semi-intact preparation that allows unrestricted siphon movements and 2) a pinned out preparation like that originally used to characterize the LE cell response properties (Byrne, Castellucci, & Kandel, *J. Neurophysiol.*37:1041, 1974). In both preparations LE cells showed maximal activation (including afterdischarge for up to 15 sec) by strong siphon pinch. LE cells displayed a very wide dynamic range to von Frey hair stimuli, sometimes responding weakly to light pressure, and showing graded activation to increasing pressures. Significant depression of mechanosensory threshold was produced by either pinching the unrestrained siphon or by testing the siphon in the pinned out preparation. Siphon pinch or siphon pinning also dramatically increased soma excitability. Water jets that activated LE cells in the pinned out preparation rarely evoked responses in the unrestrained siphon. Weak vibratory stimuli applied several cm away from the siphon never activated LE cells, while reliably activating a set of unidentified neurons in the abdominal ganglion. These results indicate 1) that LE cells can encode nociceptive information, as well as information about innocuous mechanical stimuli, 2) that receptive field injury greatly enhances peripheral and central excitability of the LE cells, and 3) in an extension of previous observations (see Cohen *et al. Soc. Neurosci. Abstr.* 17:1302, 1991), that *Aplysia* has unidentified siphon mechanoreceptors that are far more sensitive to weak mechanical stimuli than the LE cells are.

659.5

OCTOPAMINE MAKES LOCUSTS PAY ATTENTION

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Like schoolchildren, insects experience boredom, showing declining responsiveness to repetitive stimulation. However, both can be made more alert and attentive (or aroused) by suddenly presenting an additional, novel stimulus. A clear example of arousal in the locust is provided by the visual system: the habituated response of the descending contralateral movement detector (DCMD) interneuron to repetitive visual stimuli can be dishabituated (aroused) by a variety of other visual and tactile stimuli[1].

We believe octopamine mediates arousal in this system because we find its exogenous application to the locust brain and optic lobes dishabituates the DCMD, much like tactile stimuli do. The locust CNS contains 4 octopamine-immunoreactive neurons, the protocerebral medulla 4 (PM4) neurons[2], that could potentially mediate this effect. PM4 neurons project from the deutocerebrum, through the protocerebrum, and into the optic lobe. Mass spectrometry has confirmed that each PM4 cell body contains approximately 25pg octopamine[2]. Activity in PM4 neurons is increased by tactile stimulation of the locust's head or body, by auditory stimuli or by light on/off visual stimuli[2]. To provide categorical evidence that PM4 neurons can dishabituate the DCMD, we recorded extracellularly from the DCMD and intracellularly from one of the PM4 neurons. When PM4 action potentials were suppressed with hyperpolarising current, the DCMD habituated to a moving visual stimulus. However, depolarising a PM4 neuron, to produce action potentials at approximately 20Hz, significantly increased the number of DCMD action potentials per stimulus. The PM4 neurons therefore play an important role, presumably by the release of endogenous octopamine within the optic lobe, in dishabituation of DCMD by novel stimuli.

1. Rowell, C.H.F. (1971) *J. Exp. Biol.* **55**, 727-747.
2. Stern, M. et al. (1995) *J. Comp. Physiol.* (in press).

659.6

CELLULAR ANALYSIS OF HABITUATION OF THE PROLEG WITHDRAWAL REFLEX OF LARVAL *MANDUCA SEXTA*.

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Stimulation of planta hairs on an abdominal proleg of larval *Manduca sexta* evokes withdrawal of the proleg toward the body wall. The proleg withdrawal reflex (PWR) habituates with repeated deflection of one or more planta hairs (Wiel & Weeks, 1992, *Soc. Neurosci. Abstr.* **18**:942). In semi-intact preparations consisting of a proleg and the associated segmental ganglion, repeated deflection of a planta hair or electrical stimulation of its sensory neuron results in a decrease in the number of spikes evoked in the proleg motor nerve (Wood, Wiel & Weeks, 1994, *Soc. Neurosci. Abstr.* **20**:582). This decrease in evoked motor neuron activity is a neural correlate of habituation of the PWR.

Using the same preparation, we recorded intracellularly from the principal planta retractor (PPR) motor neuron during habituation training (20 brief trains of electrical stimuli delivered to a planta hair sensory neuron, at 60 s intervals). The number of spikes evoked in PPR decreased significantly over training, representing a neural correlate of habituation in a single motor neuron. Several possible underlying mechanisms were examined. The amplitude of monosynaptic EPSPs evoked in PPR by the stimulated sensory neuron did not differ significantly before and after training. PPR's resting membrane potential, input resistance and spike threshold were also measured before and after training. There were no differences between the habituation group and a control group which received only trials 1 and 20 during training. These results suggest that other mechanisms, such as changes in polysynaptic pathways in the reflex circuit, may underlie habituation of the *Manduca* PWR. Supported by NIH T32 GM07257 (DEW), R01 NS23208, K04 NS01473, the Murdock Trust and an NSF Presidential Young Investigator award (JCW).

659.7

CHARACTERIZATION OF A MORPHOLOGICALLY DISTINCT SUBSET OF SENSORY NEURONS IN THE PLEURAL GANGLION OF *APLYSIA*.

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The tail-siphon withdrawal reflex of *Aplysia* is a useful model system for studying simple forms of learning and memory. The sensory neurons mediating the reflex are located in a cluster on the ventrolateral surface of the ganglion. Most of these neurons project to the periphery through pedal nerves, including those to the head (Zhang et al. 1993). A small number projected through the cerebral-pleural connective. Because of their unusual projection, we wanted to confirm that these neurons were sensory in function. Members of this subset (n=20) were identified by stimulating ipsilateral cerebral nerves. Their location on the rostral edge of the cluster corresponded to the subset of sensory neurons labeled by backfilling the cerebral-pleural connective. Tactile stimulation of the head skin elicited action potentials without prepotentials. Hyperpolarization of the cell body reduced the amplitude of the action potentials. Most of these sensory neurons had receptive fields on the anterior tentacle (n=17), and a few had receptive fields on the posterior tentacle (n=2) and lips (n=1). Physiologically-identified cells were injected iontophoretically with 2% dextran tetramethylrhodamine. All of these neurons projected out both the cerebral-pleural and pleural-pedal connectives. Some of these neurons were bipolar (n=8). In 10 preparations, axons could be traced out cerebral nerves (C1=1, C2=7, C4=2). In 2 preparations, a contralateral branch arose within the cerebral ganglion.

Our results suggest that a subset of sensory neurons located on the rostral edge of the pleural sensory cluster are primary mechanoreceptors for the head. These neurons are morphologically distinct in that they are either bipolar or bifurcate within the pleural ganglion. Functionally, they may be more closely related to sensory neurons in the J and K clusters of the cerebral ganglion, which also project to the pedal and pleural ganglia, than to other sensory neurons in the pleural cluster.

659.8

CELLULAR CORRELATES OF LONG-TERM SENSITIZATION IN *APLYSIA*.

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Long-term sensitization is an important form of learning exhibited by the tail-siphon withdrawal reflex. Previous work demonstrated that long-term sensitization was lateralized, and was correlated with changes in the membrane properties of tail sensory neurons (Scholz and Byrne, 1987). In this study, we extended these observations to include properties of both sensory and motor neurons innervating the tail, as well as the synaptic connections between them.

Animals were trained as described previously (Scholz and Byrne, 1987). Enhancement of siphon withdrawal was greater on the sensitized side of the animal than on the contralateral control side (186 ± 14% vs. 121 ± 7%, $P < 0.0001$). Training increased the excitability of sensory neurons from the sensitized side of the animal. A depolarizing current pulse (2 nA, 1 sec) elicited 14.3 ± 1.4 spikes on the sensitized side compared with 7.5 ± 1.2 spikes on the control side ($P < 0.001$). Moreover, the afterdepolarization following the pulse was enhanced in sensitized ganglia compared with controls (2.7 ± 0.8 mV vs. 0.7 ± 0.3 mV, $P < 0.03$). Resting membrane potential and input resistance were unaffected. Training had no significant effects on properties of tail motor neurons, including resting membrane potential, input resistance and spike threshold. Synaptic currents elicited by a single action potential in a sensory neuron were calculated by dividing the amplitude of the evoked EPSP by the input resistance of the motor neuron. Training enhanced the calculated synaptic current (1.85 ± 0.33 nA vs. 0.92 ± 0.23 nA, $P < 0.03$).

In these experiments, long-term behavioral training produced a lateralized enhancement of sensory neuron excitability, which is consistent with the change in net outward currents described previously (Scholz and Byrne, 1987). Synaptic transmission between sensory and motor neurons mediating tail withdrawal was also enhanced in the trained side of the animal. Therefore, these experiments confirm that the same cellular changes induced *in vitro* (Zhang et al, 1994) are correlated with long-term sensitization *in vivo*.

659.9

CHANGES IN CALMODULIN (CAM) AND ROLE OF CA^{2+}/CAM-DEPENDENT PROTEIN KINASE II IN SYNAPTIC FACILITATION IN *APLYSIA*.

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Experiments using *in vitro* translation of mRNA indicated that the level of mRNA for calmodulin (CaM) was increased by an *in vitro* analogue of sensitization training in *Aplysia* (Zwartjes, et al., 1992). This raised the possibility that CaM and Ca^{2+}/calmodulin-dependent protein kinase II (CaMK) may play a role in the induction and maintenance of short- and/or long-term facilitation (STF and LTF). Indeed, we previously found that KN62, an inhibitor of CaMK, reduced 5-HT-induced STF in sensorimotor synapses in the pleural-pedal ganglia (Nakanishi *et al.*, 1994). The present studies have extended these findings. First, using ribonuclease protection assays, we confirmed the increase of CaM mRNA in sensory neurons (SN) induced by 5-HT, and also by behavioral training, which induced long-term sensitization (see Lee et al., this volume). Second, we examined LTF in isolated pleural-pedal ganglia. The amplitudes of EPSPs at identified sensorimotor connections were measured before and 24 hr after nerve stimulation, an analogue of long-term sensitization training. Neither KN62 nor KN04 (an inactive form of KN62), applied during stimulation, blocked the induction of LTF. Third, to test the possible role of CaMK in the maintenance of LTF, we continued the experiment by applying KN62 after the 24 hr tests, and testing the EPSPs again 15 min later. KN62 did not block the expression of LTF. Finally, we examined the effects of KN62 on 5-HT-induced spike broadening and increases in excitability in SN. KN62 was applied to isolated SN soma after 3 baseline tests (ISI=5 min) and 15 min before the application of 5-HT. KN62 by itself induced a slight increase in the duration of the action potentials relative to KN04, but did not block 5-HT-induced broadening. In addition, KN62 had *no effect* on either basal excitability or the 5-HT-induced increases in excitability. These data suggest that a spike duration independent (SDI) process contributes to STF and that CaMK plays a role in this SDI process. Although CaMK does not appear to be necessary for LTF, calmodulin may act through mechanisms other than CaMK.

659.10

A *TOLLOID*-LIKE GENE IS REGULATED IN *APLYSIA* NEURONS BY TREATMENTS THAT INDUCE LONG-TERM MEMORY.

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Long-term facilitation of sensory-motor connections of *Aplysia* is produced by long-term sensitization training or procedures that mimic that training. The long-term effects are dependent on protein and mRNA synthesis (Montarolo et al., 1986). Recently, we began to use differential display reverse transcription PCR (DDRT-PCR) to identify genes whose expression is regulated by long-term training procedures. We found several mRNAs whose levels appeared to be regulated immediately after treatment of isolated pleural-pedal ganglia with 5-HT for 1.5 hr. One of these mRNAs was selected for further study. The labelled PCR fragment affected by 5-HT was reamplified and cloned. To confirm the change in mRNA of this clone observed using DDRT-PCR, ribonuclease protection assays (RPA) were performed to examine mRNA from pleural-pedal ganglia treated with 5-HT for 1.5 hr. 5-HT significantly increased the levels of mRNA for this clone in the ganglia. Moreover, this mRNA was also increased in tail sensory neurons isolated from the ganglia. Finally, we found that this mRNA level in sensory neurons was also increased by long-term behavioral training.

Aplysia head ganglia cDNA libraries were screened to identify the gene affected by 5-HT. Eight separate positive clones were isolated with different insert sizes (1.5–4.0 kb). Sequence analysis showed that the *Aplysia* gene affected by 5-HT is 41-45% identical to a developmentally regulated gene family including *Drosophila tolloid* gene and human bone morphogenetic protein 1 (BMP-1). Both *tolloid* and BMP-1 appear to function as zinc proteases to activate TGF-β like molecules. The finding that a *tolloid*-like gene is regulated by 5-HT is important because a large number of studies have shown that synaptic morphological changes accompany the formation of memory in *Aplysia* and in other animals. The *Aplysia tolloid*-like protein could be involved in regulating the morphological changes that are associated with long-term facilitation and other examples of synaptic plasticity.

659.11

CLONING AND CHARACTERIZATION OF A NOVEL GENE REGULATED BY TREATMENTS PRODUCING LONG-TERM FACILITATION IN *APLYSIA* SENSORY NEURONS. K.MacPhee, R.Homayouni, X.Ren, H.West, J.H.Byrne#, and A.Eskin*. Dept. of Biochem. and Biophysical Sci., Univ. of Houston,77204. Dept. of Neurobiology and Anatomy#, Univ. of Texas Med. School, Houston, TX 77225.

Long-term memory in *Aplysia* requires protein and RNA synthesis and can be induced by exposing neural tissue to serotonin (5-HT) (Montarolo, 1986). Using in vitro translation of mRNA and two-dimensional polyacrylamide gel electrophoresis of the protein products, we previously found three proteins, calmodulin, phosphoglycerate kinase and an unidentified one (protein 3) whose mRNA levels were increased in response to treatments that mimic sensitization training (Zwartjes et al, 1992; Eskin et al, 1993). To identify protein 3, we screened a Lambda Zap library of *Aplysia* ganglia with a 132 bp PCR probe amplified using degenerate oligonucleotides designed from the peptide sequence. A 1750 bp cDNA clone, which contained the DNA sequence that codes for the original peptide (58 amino acids), was isolated. An amino acid sequence of 210 residues deduced from the middle of the cDNA clone of protein 3 yielded no similar sequences in a search of Genbank. Approximately 90% of the cDNA clone has been sequenced in one direction. We are currently sequencing the opposite strand. To confirm the change in levels of mRNA we initially observed, ribonuclease protection assays have been used to measure mRNAs. Levels of protein 3 mRNA in pleural sensory neurons were increased by 88±30%, n=5 when ganglia were exposed to 5-HT for 1.5h. Moreover, long-term behavioral training also increased protein 3 mRNA (48±11%, n=6) in sensory neurons.

659.12

ACQUISITION AND RETENTION OF HABITUATION OF THE OLFACTO-CARDIAC RESPONSE IN *PROTOPHORMIA TERRAENOVAE*. A.M. Angioy*, P. Casula, P. Muroni, N. Piroddi, C. Reali, I. Tomassini Barbarossa. Department of Experimental Biology, General Physiology, University of Cagliari, Viale Poetto 1, 09126 Cagliari, Italy.

Olfactory stimulation of antennal receptors can release the cardiac response of premature heart-beat reversal in the blowfly. Repeated stimulation with 1-hexanol vapors can iteratively evoke the response or lead to response habituation depending on the efficacy of the chemical concentration in inducing behavioral responses of rejection as well.

By simultaneously recording heart activity and sensory input on intact flies, we examined the dynamics of acquisition and retention of response habituation as a function of stimulus intensity and interstimulus interval (ISI). The response habituates faster to low intensity stimulation repeated at the shortest and longest ISI. Duration of habituation retention is directly related to stimulus intensities adopted, and it ranges between 30' and 120' mean values. The response to olfactory stimulation is promptly restored in habituated flies, i.e. dishabituation takes place, following intense stimulation of mechano-taste receptors on the labellar area.

659.13

LONG-TERM HYPEREXCITABILITY OF *APLYSIA* SENSORY NEURONS: INTERACTIONS OF AXOTOMY AND 5-HT. X. Liao, J. D. Gunstream, T. M. Grogean, and E.T. Walters*. Department of Integrative Biology, Univ. of Texas - Houston Medical School, TX 77030.

Both repeated 5-HT application to sensory neurons (SNs) in dissociated cell culture (Dale & Kandel, *J. Neurosci.* 7:2232, 1987) and close axotomy of SNs in organ culture (Gunstream *et al., J. Neurosci.* 15:439, 1995) enhances soma excitability 1 day later. To begin to explore potential interactions between signal pathways activated by axon injury and by 5-HT, we have examined the effects of 5 applications of 5-10 μM 5-HT on SNs axotomized different distances from the soma 1-2 hr earlier. 5-HT treatment enhanced the excitability (tested 20-30 hr later) of SNs whose axons had been crushed 10-20 mm from the soma (8.6 ± 0.7 spikes after 5-HT vs. 6.0 ± 1.1 spikes in contralateral controls, p=0.02; n=7 animals). This distance is short enough to allow retrograde transport of axonal injury signals to the soma within 20 hr. In contrast, 5-HT application had little effect on the excitability of SNs in which the distance to the axonal crush was too long (>40 mm) for such signals to reach the soma by this time (4.5 ± 1.2 vs. 4.2 ± 0.7 spikes, n=5). [However, 1 of these 5 "long nerve" preparations did show a significant within-animal effect of 5-HT.] Repeated application of 50 μM 5-HT had no obvious effect in the "long nerve" preparation (3.5 vs. 3.5 spikes, n=2 animals). These results suggest that axonal injury signals may permit 5-HT-induced long-term hyperexcitabilty to be expressed. Injury signals produced by extremely close axotomy (excising the SN somata) were not sufficient to permit 5-HT-dependent soma hyperexcitability (5.9 ± 1.3 after 5-HT vs. 6.8 ± 0.7 spikes, n=5 animals), indicating that interactions between 5-HT and axotomy signals are complex and depend upon the site of axonal injury.

659.14

AXOTOMY-INDUCED HYPEREXCITABILITY OF *APLYSIA* SENSORY NEURONS REQUIRES PERIPHERAL CALCIUM. J. D. Gunstream*, G.A. Castro and E.T. Walters. Department of Integrative Biology, Univ. Texas - Houston Medical School, TX 77030.

Nerve injury in *Aplysia* leads to long-term hyperexcitability of the central somata of sensory neurons (SNs), which is triggered (at least in part) by retrograde transport of axonal injury signals to the soma (Gunstream *et al., J. Neurosci.* 15:439, 1995). To begin to define initial cellular signals of axonal injury we used an *in vitro* preparation in which peripheral nerves containing axons of VC SNs could be treated selectively. 24-30 hr after proximal nerve crush, a 1 sec depolarizing pulse at 2.5x threshold elicited 6.8 ± 0.8 spikes in axotomized SNs, whereas paired, contralateral SNs whose axons were left in 0 Ca^{2+} (nominally) or 0 Ca^{2+} and EGTA displayed only 3.8 ± 0.3 spikes ($p<0.001$, n=29 cell pairs). A solution containing 100 μM Ca^{2+} did not prevent hyperexcitability (7 spikes vs. 6.5 spikes in normal Ca^{2+}, n=8 cell pairs). Elevation of Ca^{2+} alone does not appear sufficient to trigger hyperexcitability since calcium ionophores (100 μM ionomycin or calcimycin) applied to long nerves (which do not exhibit hyperexcitability within 30 hr) had no significant effect (4.0 spikes vs. 4.4 spikes without ionophores, n=24 cell pairs). However, an initial study using 100 μM BAPTA-AM (which is membrane permeant) suggests that an elevation of intracellular Ca^{2+} may be necessary for triggering long-term hyperexcitability. How Ca^{2+} exerts its necessary role(s) is not known. Preliminary experiments have revealed no consistent effects on hyperexcitability of bathing the nerve crush site with several nonspecific inhibitors protein kinases (K-252a, staurosporine, and KN-62; 5-20 μM each).

659.15

ACETYLCHOLINE INDUCES INK SECRETION FROM ISOLATED INK VESICLES IN *APLYSIA*. T.L. Ross*, J. Prince and T.G. Nolen Dept. of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124.

The secretion of ink provides *Aplysia* with an active chemical defense against predators (Nolen *et al*, ICP 176:239-254). Ink pigments are extracted from the phycobilisomes of red algal rhodoplasts. An ultrastructural analysis combined with immuno-gold labeling of phycoerythrin (the main ink pigment) suggests that it is released by rhodoplast catabalism in the hepatopancreas. Soluble pigments are taken up by granulate cells within the ink gland via coated vesicles and then packaged in large (50-1000μm) secretory vesicles that develop from single uni-nucleate cells. Activation of muscles surrounding each vesicle forces ink through a valved duct to the gland's surface.

Ink secretion is centrally mediated by a neural circuit within the Abdominal ganglion (Abd. g.) [Carew and Kandel, 1977]. We present evidence that the peripheral control of vesicle activation is cholinergic: Isolated individual ink vesicles were dissected from undisturbed ink glands and assayed using an ascending conc. series of ACh dissolved in high divalent cation ASW. ACh induced ink release from 33 of 38 vesicles compared to 1 of 9 controls ($p<0.0005$) (N=3 animals). At least 75% of the vesicles released ink at ACh concentrations ≤ 0.5mM (median = 0.25mM). Koester & Kandel (1977) report that the identified neurons in the Abd. g. thought to be responsible for generating the inking response ($L14_{A-C}$) are non-cholinergic. Our evidence for cholinergic activation of individual ink vesicles implies the existence of yet unidentified cholinergic central and/or peripheral ink motoneurons. This is further indicated by experiments where the peripheral nerves containing the axons of $L14_{A-C}$ were cut. Following recovery, ink secretion was still observed if other peripheral nerves were left intact (N=5). These results suggest that other neurons in addition to $L14_{A-C}$ are sufficient for inking. Currently we are employing intracellular staining, tract tracing, immunohistochemical and pharmacological techniques to identify central and peripheral neurons of the ink circuit. [Supported by an NIMH BRSG to TGN and JP]

660.1

RAPID CONDITIONED CHANGES IN TASTE REACTIVITY RESPONSES: COMPARING TASTE AND ODOR CUES. K.-P. Ossenkopp*, B. Navarro, L. A. Eckel and S. N. D. A. Clarke. Neuroscience Program and Department of Psychology, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

Conditioned flavour aversions (CFAs) can be produced by pairing ingestion of a novel food with exposure to a toxin, such as LiCl. Using a rapid, within session, conditioning paradigm, the effects of pairing a novel taste (saccharin), odor (almond), or taste-odor mixture (saccharin-almond) with LiCl treatment, on conditioned changes in taste reactivity responses (TRRs) were examined. On 2 occasions male rats were injected with NaCl or LiCl (3 meq/kg, i.p.) placed in an observation box for 30 min, and given 7 brief (30 sec) intraoral infusions (0.78 ml/min) of a sodium saccharin (0.2%), almond odor (2%), or saccharin-almond mixture dissolved in distilled water, at 5 min intervals. Relative to the NaCl control rats, the LiCl treated subjects exhibited significant ($ps < 0.01$) conditioned reductions in ingestive TRRs and increased levels of passive drip. When tested with saccharin or almond alone in a CFA test phase (no injections), conditioning to the taste or odor cue was found to be equally strong. The present results provided no evidence for a taste-potentiated odor conditioning effect. (Supported by NSERC and MRC of Canada).

660.2

EXCITOTOXIC LESIONS OF THE RAT LATERAL HYPOTHALAMUS: EFFECTS ON TASTE AVERSION. J.M. Scollon, M.P. Latimer and P. Winn (SPON: Brain Research Association) School of Psychology, Univ. St Andrews, Fife, Scotland KY16 9JU.

Excitotoxic lesions of lateral hypothalamus (LH) produce small reductions in intake; no motor impairments; continued ability to regulate intake after addition of glucose or salt to the diet; normal responding to food and water deprivation (including prandial drinking). LH lesioned rats also show normal motivation measured by responding for food on progressive ratio schedules. There are though always impairments in responding to physiological challenges such as hypertonic saline. Excitotoxic lesioned LH rats also respond as controls to adulteration of the diet with quinine or saccharin, indicating normal taste processing. Taste aversion conditioning (the association of a specific taste with experimentally produced malaise) has not been investigated. To examine this, bilateral LH (2.0 µl 0.09M NMDA in phosphate buffer) and control (2.0 µl phosphate buffer) rats were water deprived for 19h/day for 3 days. On the fourth day rats were given 0.15% saccharin solution instead of water for 30 min. Intake did not differ between lesioned and control rats. Immediately after, half the LH and half the controls were given 20ml/kg 0.15M LiCl; the remainder had an equal volume of NaCl. Behavior was observed for 30 min post-injection. All LiCl treated rats showed a characteristic "lying-on-belly" reaction. 3 days after this rats had a two-bottle drinking test: one bottle contained 0.15% saccharin solution, the other tap water. Intake of saccharin solution was suppressed in both LH and control rats which had previously had LiCl injections; injection of NaCl had no effect. In further tests, drinking in response to hypertonic saline was significantly suppressed in LH rats compared to controls. These data indicate that LH lesioned rats which characteristically fail to respond as controls to injections of hypertonic saline perform normally in tests of taste aversion.

660.3

AMYGDALA LESIONS ATTENUATE PICA ELICITED BY LITHIUM CHLORIDE INJECTIONS BUT NOT BY FOOD DEPRIVATION IN RATS. D. Mitchell*[1] and [2]S. Maren. [1]Dept. of Psychol., Univ. of So. Calif., Los Angeles, CA 90089, [2]Dept. of Psychol., Univ. of. Calif., Los Angeles, CA 90024.

The amygdala is known to modulate a wide spectrum of visceral phenomena including gastric motility, secretions, pathology, and gastrointestinal malaise. In previous research, we have demonstrated that pica, the consumption of non-nutritive substances, is a useful animal model for quantifying unpleasant visceral sensations produced by a variety of treatments such as food deprivation and lithium chloride injections. We here report two experiments investigating the effects of bilateral electrolytic (Experiment 1) and ibotenic acid (Experiment 2) amygdala lesions on food deprivation- and illness-elicited pica. In Experiment 1, lesioned and sham-operated rats were maintained with food, water, and kaolin (non-nutritive clay) continuously available throughout the testing sequence, except for the initial food deprivation test during which the food was removed from all rats for 24 h. Two weeks following surgery and three days following the food deprivation test, half of the sham rats and rats with amygdala lesions were administered lithium chloride (0.15 M, 20 ml/kg, i.p.) every third day for a total of five treatments. The remaining sham rats were treated identically except that they were administered an equivalent volume of isotonic saline (0.15 M) on each of the five treatment days. Though all three groups engaged in comparable amounts of deprivation-elicited pica, amygdala lesions eliminated the robust increase in illness-elicited pica apparent in shams given repeated lithium chloride treatments. Saline controls did not engage in pica on any treatment day. A similar pattern of results was obtained in Experiment 2 with ibotenic acid lesions in the amygdala. These results reveal that neurons in the amygdala mediate behavioral responses to the visceral malaise induced by lithium chloride, but not by food deprivation, and they further demonstrate the utility of pica as an effective tool in studying the psychophysics of subjective visceral sensation.

660.4

THE EFFECT OF INSULAR CORTEX LESIONS ON LITHIUM CHLORIDE INDUCED BEHAVIORAL RESPONSES AND CONDITIONED TASTE AVERSION. P. A. Bryant*, M. A. Norris and I. S. McGregor Dept. Psychology, University of Sydney, NSW, 2006, Australia.

Lithium chloride (LiCl) produces nausea and emesis in humans and is frequently used to induce conditioned taste aversions (CTA) in animals. In rats, LiCl induces a series of unconditioned responses including reduced food intake, hypothermia and decreased motor activity as well as "lying on belly" (LOB), believed to be a postural index of malaise.

The insular cortex (IC) receives taste and visceral afferents from thalamic and brainstem nuclei and has been implicated in the acquisition and retention of CTAs. Previous research from our laboratory has demonstrated increased expression of the c-fos gene in the IC of rats following LiCl injection and following exposure to conditioned and unconditioned taste stimuli.

Water-deprived IC lesioned and sham lesioned rats were given 30min access to 0.1% saccharin solution and then immediately injected (i.p.) with LiCl (0.15M, 20 mg/kg). They were assessed for unconditioned effects of LiCl (hypothermia, anorexia and LOB) for one hour. Starting two days later, they were given four daily one-bottle test sessions (30min) where intake of saccharin was measured. IC lesions had no effect on any of the unconditioned responses to LiCl, with sham and lesioned animals exhibiting a similar level of LOB, aphagia and hypothermia. IC lesions also had no effect on neophobia to the 0.1% saccharin. However, IC lesioned animals showed significantly faster extinction of a CTA to saccharin.

This suggests that although the insular cortex is clearly activated by LiCl (as shown by c-fos expression) it does not mediate the unconditioned effects of LiCl. Rather it may be that the insular cortex is involved in the associational mechanism whereby taste and illness are paired.

660.5

ALTERED INDUCTION OF c-FOS IN THE CENTRAL NUCLEUS OF THE AMYGDALA (CeN) CORRELATED WITH CONDITIONED TASTE AVERSION EXPRESSION. T.A.Houpt*, R.A. Berlin, and G.P. Smith E.W.Bourne Behav. Res.Lab.,Dept.Psychiatry, Cornell Univ.Med.Coll., White Plains, NY 10605.

The induction of c-Fos-like immunoreactivity (c-FLI) in the intermediate nucleus of the solitary tract (iNTS) is a neuronal correlate of conditioned taste aversion (CTA) expression (Houpt et al., 1994; Swank et al., 1994). Decerebrate preparations have demonstrated that intact connections between the hindbrain and forebrain are required for behavioral expression of a CTA. The CeN is a candidate forebrain site required for CTA expression. We quantified c-FLI induction after CTA expression in the CeN as a marker of neuronal activation during CTA expression.

Adult male rats were implanted with sublingual intraoral catheters. Conditioned rats received intraoral infusions of 5% sucrose (6ml/6min) paired with a LiCl injection (12ml/kg, 0.15M ip) 3 times over 1 week. Unconditioned controls received LiCl and sucrose on alternate days (non-contingently) so that no CTA against sucrose was acquired. Two days after the last pairing, rats recieved an unpaired infusion of 5% sucrose (6ml/6min). One hr later, rats were sacrificed and processed for c-FLI. Positive cells were counted within a 0.25 mm radius circle centered on the CeN in conditioned and unconditioned rats. An intraoral infusion of sucrose induced more c-FLI-positive cells in the CeN of conditioned rats (68.3 ± 13.1, n=3) than a sucrose infusion did in unconditioned rats (21.0 ± 2.6, n=3). An acute LiCl injection (0.15M, 12ml/kg) also induced large numbers of c-FLI positive neurons in the CeN (155.0 ± 9.7, n=3). All 3 groups were significantly different from each other (p's <0.005).

Thus the CeN responds differently to an intraoral sucrose infusion at the level of gene expression after acquisition of a CTA against sucrose. The observation of altered c-FLI induction in the CeN complements and parallels our observation of altered c-FLI induction in the iNTS. The CeN has reciprocal connections with many brain regions, including the medial iNTS. This raises the possibility that the induction of c-FLI in the iNTS during CTA expression is causally linked to activation of the CeN.

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660.6

VARIATION IN AVOIDANCE OF BITTER COMPOUNDS BY RODENTS AND BIRDS. S.A. Wager-Pagé* and J.R. Mason, USDA/APHIS/ADC/DWRC, c/o Monell Chemical Senses Center, Philadelphia, PA 19104.

Quinine hydrochloride (QH) and denatonium benzoate (DB) are non-toxic, chemically dissimilar, bitter tasting compounds. The perceived toxicity of bitter compounds can modify dietary selection by rodents and birds. We evaluated responses of two rodent species, deer mice, Peromyscus maniculatus and, prairie voles, Microtus ochrogaster, and an avian species, European starlings, Sturnis vulgaris to the bitter taste of QH and DB. The effect of adulteration of a preferred food, apples, by soaking in QH or DB (0.01 & 0.1% v/v) solutions was evaluated during 2 hr, 2 choice feeding trials. Both DB and QH (0.01 & 0.1% v/v) decreased apple intake in mice, voles, and starlings $P<0.05$. Voles exhibited greater avoidance of QH (0.1%) than DB (0.1%) selecting 27.8% of their intake from the QH adulterated apples compared to 39.8% from DB treated apple pieces in separate trials, $P<0.05$. Voles avoided QH to a greater extent than either mice or starlings, $P>0.05$. European starlings were less sensitive than either deer mice or prairie voles to these bitter substances requiring higher concentrations of QH and DB to inhibit intake. Despite intraspecies variation in responsiveness, both rodents and birds avoided QH and DB adulterated apples. Therefore, these compounds may be useful in wildlife management of a diverse number of vertebrate species. (This work was supported by a cooperative agreement between USDA/APHIS/ADC/ DWRC and Monell Chemical Senses Center.)

660.7

FOREBRAIN CONTRIBUTION TO THE CONDITIONED EXPRESSION OF c-FOS IN NTS FOLLOWING TASTE AVERSION LEARNING. Glenn E. Schafe, Randy J. Seeley, & Ilene L. Bernstein*. Department of Psychology, University of Washington, Seattle, WA 98195

The induction of c-Fos-like immunoreactivity (c-FLI) in intermediate NTS following re-exposure to a taste (CS) which has been paired with LiCl appears to be a reliable cellular correlate of the behavioral expression of a conditioned taste aversion (CTA). This pattern of c-FLI is similar to that seen following administration of the US drug (LiCl). The present studies employed a variant of the chronic decerebrate rat preparation to explore whether circuitry intrinsic to the brainstem is sufficient to support the induction of c-FLI in NTS following both administration of the US drug and re-exposure to the CS taste. In the first experiment, chronic hemidecerebrate rats, which have a unilateral supracollicular brain transection, were injected with either LiCl or NaCl. Hemidecerebrates in the second study were re-exposed to a saccharin CS which had been either paired or unpaired with LiCl. Results indicated that the bilateral induction of c-FLI following LiCl administration was unaltered by the transection, while expression of c-FLI following exposure to the CS taste was evident only on the side of the brain which retained neural connections with the forebrain. These findings indicate that forebrain connections are necessary for the induction of c-FLI in NTS during the behavioral expression of a CTA, but not for that following administration of LiCl. Thus, in the CTA paradigm, two distinct neural pathways appear to mediate the response to the US (LiCl) and CS (taste).

660.8

ROLE OF THE AREA POSTREMA IN CISPLATIN-INDUCED TASTE AVERSION LEARNING IN RATS. B. M. Rabin*. Dept. of Psychology, Univ. of Maryland Baltimore County, Baltimore, MD 21228.

A conditioned taste aversion (CTA) is produced when ingestion of a novel food is paired with a toxin, such that the organism avoids ingestion of the food at a subsequent presentation. CTA learning following treatment with many toxins in dependent upon the integrity of the area postrema (AP), the chemoreceptive trigger zone for emesis. Two sets of experiments examined the role of the AP in the acquisition of a cisplatin-induced CTA and its relationship to a CTA produced by lithium chloride (LiCl).

Injection of cisplatin (3-5 mg/kg, ip) produced a significant test day reduction in the intake of the conditioned stimulus (10% sucrose solution) using a two-bottle test. However, interpretation of these results was confounded by the observation that the injection of cisplatin also produced a reduction in total fluid intake. AP lesions prevented both the acquisition of the cisplatin-induced CTA and the reduction in fluid intake. Preexposing rats to 3 unpaired injections of LiCl (3 mEq/kg, ip) prevented cisplatin-induced CTA learning. Preexposure also prevented the cisplatin-induced reduction in fluid intake.

The results support the hypothesis that the integrity of the AP is necessary for the acquisition of a cisplatin-induced CTA, and that the mechanisms by which cisplatin leads to the acquisition of a CTA are similar to those of LiCl.

660.9

TASTE AND OTHER ORAL RESPONSES ASSOCIATED WITH OVEREATING. P.J.Geiselman*, C.F.Smith, D.A.Williamson, V.Broussard, R.Plum, L.Womble, V.Nguyen, C.Carlton, N.Baker, G.A.Bray, C.Champagne, and D.H.Ryan. Dept. Psychol. & Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808.

Caloric intake can be largely determined by the palatability of food and many highly palatable foods are high in sugar and fat content. The present study was conducted to determine how taste and other oral responses to a high sugar/high fat preload may account for overeating. The Three-Factor Eating Questionnaire was used to identify four groups of women according to history of dieting (High or Low Restraint) and tendency to binge eat (High or Low Disinhibition). Half of the subjects were given a high sugar/high fat preload of chocolate pudding to eat and then were presented with a standard pasta product and told that they could eat as much or as little as they wished. Control subjects were given the pasta only. Regardless of patterns of Restraint or Disinhibition, subjects in the Preload condition ate twice as many calories as did subjects in the control condition. Data will be presented to show how taste and other oral responsivity to the high sugar/high fat chocolate preload are associated with overeating in each of the four groups.

660.10

FAT PAD-SPECIFIC RESPONSES TO HIGH-FAT OR HIGH-CARBOHYDRATE DIET IN DIABETIC RATS. M.F. Wiater*, C.A. Matson, M. Chavez, and S.C. Woods. Department of Psychology, University of Washington, Seattle, WA 98195.

Retroperitoneal white adipose tissue (RWAT) and mesenteric white adipose tissue (MWAT) are more responsive than epididymal white adipose tissue (EWAT) to changes of food intake. Neural and/or humoral factors could be involved. To differentiate these possibilities, we changed the humoral environment by use of streptozotocin (STZ). Diabetic rats (STZ 60 mg/kg; glucose> 400 mg/dl) were divided into two weight- matched groups and fed a high-fat diet (HF; n=15; fat = 43%, carb=19% by weight of diet) or a high-carbohydrate diet (HC; n=13; fat=6%, carb=70% by weight of diet). Non-diabetic controls (n=6) weighed 339 ± 6 g. Diabetes resulted in hyperphagia on the HC diet, but normophagia on the HF diet. Beginning body weights of HF (348 ± 8 g) and HC (345 ± 9 g) were not different. At sacrifice, 4 wks, body weights of HF (347 ± 12 g) and HC (345 ± 11) were also not different, nor were they different from beginning body weights. HC combined fat pad mean weight (5.0 ± 0.6 g) was reduced significantly ($p<0.05$) compared to HF combined fat pad mean weight (9.5 ± 0.8 g). HF rats had comparable EWAT (3.4 ± 0.3 g) and RWAT (3.7 ± 0.5 g) weights. However, HC rats had smaller RWAT (1.4 ± 0.3 g) than EWAT (2.2 ± 0.3 g). In controls, RWAT (4.4 ± 0.3 g) was heavier than EWAT (3.4 ± 0.2 g). Thus, in non-diabetic controls RWAT weighs more than EWAT; in HF, RWAT weighs the same as EWAT; and, in HC, RWAT weighs less than EWAT. MWAT weighs less than EWAT. MWAT mean weight in HC rats was 1.3 ± 0.1 g and in HF rats was 2.1 ± 0.2 g. These results suggest that RWAT is more responsive to dietary differences in diabetic rats than MWAT or EWAT, and that humoral factors are probably important in its control.

660.11

HYPERINSULINEMIA IN HYPERPHAGIC RATS WITH POSTERODORSAL AMYGDALOID LESIONS. B. M. King*, J. T. Cook, and M. F. Dallman. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148, and Dept. of Physiology, Univ. of California, San Francisco, CA 94143.

Recent studies have found that lesions of the posterodorsal amygdala in female rats result in hyperphagia and excessive weight gain (King et al., 1993, 1994). The animals gain as much as 100 g in 20 days. In the present study, female rats with either sham or posterodorsal amygdaloid lesions were maintained on a restricted feeding schedule for five days and then were given food ad libitum for 15 days. The rats with amygdaloid lesions gained a mean of 52 g during the final 15 days, compared to 14 g for controls. Plasma insulin levels were significantly elevated ($p < .001$) in the rats with amygdaloid lesions both during food restriction and at the end of the ad libitum feeding period. Basal corticosterone and ACTH levels were unaffected by the lesions. These results suggest that the effects of posterodorsal amygdaloid lesions on feeding behavior and body weight may be mediated, in part, by a change in metabolic responses.

660.12

TEMPORAL, BUT NOT SENSORY, LEARNED SIGNALS FOR MEAL INITIATION ARE ASSOCIATED WITH TRANSIENT DECLINES OF BLOOD GLUCOSE IN THE RAT. H.P. Weingarten*, F.J. Smith, L.A. Campfield and D. Elston. Department of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

Rats that have learned to associate cues with food delivery reliably initiate meals upon subsequent exposure to these learned signals for meal initiation. We explored whether the transient decline of blood glucose that is causally related to *spontaneous* meal initiation was also evident in cases of *conditioned* meal initiation. Over a 12 day period, male rats were conditioned to associate a tone-light CS+, presented at 3 hr intervals, with food and the opportunity for ingestion by providing six such signalled meals per day. After conditioning, rats were maintained ad libitum except for approximately 3 hr food deprivation imposed before tests examining the effects of the CS+ on meal initiation and blood glucose. Although the CS+ reliably elicited food anticipatory behavior and meal initiation, its presentation did not result in any systematic change of blood glucose, as measured with continuous withdrawal on-line blood glucose monitoring. However, measurement of blood glucose during the intermeal interval revealed the presence of a transient decline of blood glucose at times that, as a result of the conditioning schedule, rats had learned to anticipate a meal. The parameters of this blood glucose dynamic were similar to the premeal decline of blood glucose associated with the initiation of spontaneous meals. Control rats that had not been provided with an opportunity to learn that meals were provided every 3 hr showed no analogous changes in blood glucose concentration during the intermeal interval. These studies further define the occasions under which meal initiation is associated with transient declines of blood glucose and suggest that this dynamic may be related to meals initiated in response to endogenous signals as opposed to meals instigated by external signals such as exteroceptive CS+'s or the presence of palatable foods.

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660.13

LATERAL PARABRACHIAL NUCLEUS (lPBN) BLOCKADE ATTENUATES SALINE INTAKE BY RATS WITH LESIONS OF THE AREA POSTREMA AND ADJACENT NUCLEUS OF THE SOLITARY TRACT (AP/mNTS-lesion). T. Wang* and G.L. Edwards. Dept. of Physiol. & Pharmacol., Coll. of Vet. Med., Univ. of Georgia, Athens, GA 30602

Previous studies indicate that 3 h ad libitum saline intake as well as intake after sodium depletion by rats with AP/mNTS-lesions is greater than sham-lesioned rats (Am. J. Physiol. 264:R1242, 1993). Additionally, rats with AP/mNTS-lesions consume increased amounts of highly palatable food (Physiol. & Behav. 32:923, 1984). Electrolytic lesions of the lPBN block or reverse the overingestion of palatable food observed in AP/mNTS-lesioned rats if the lPBN-lesion precedes the AP/mNTS-lesion (Am. J. Physiol. 256:R306, 1989). The present study examined the effect of lidocaine blockade of the lPBN on saline intake in AP/mNTS-lesioned and sham-lesioned rats. All animals were implanted with bilateral cannulae in the lPBN. Bilateral injection of lidocaine into the lPBN significantly attenuated 2% saline intake in AP/mNTS-lesioned rats after sodium depletion. There was no effect of lPBN lidocaine on depletion-induced intake in sham-lesioned rats. This study suggest that lPBN plays an important role in the enhanced sodium appetite observed in rats with AP/mNTS lesions. (Supported by NIH DK 42533)

660.14

WATER LOADS DURING SODIUM DEPLETION FACILITATE SALT APPETITE IN SFO-LESIONED RATS. E. M. Starbuck, J. R. Lane, & D. A. Fitts*. Dept. of Psychology, University of Washington, Seattle, WA, 98195.

Reports from this laboratory suggest that the subfornical organ (SFO) is not important for angiotensin II (ANG II) -mediated salt appetite. However, two recent reports by others show that SFO lesions do acutely reduce salt intake after sodium depletion. One concluded, in apparent contradiction to our findings, that blood-borne ANG II may activate receptors in the SFO to elicit salt appetite. These reports also noted that the SFO-lesioned rats drank much less water acutely in response to the diuresis than sham-lesioned rats. The present study examined salt appetite in SFO-lesioned rats after water gavage to determine whether the lesion or the underhydration caused the decreased salt appetite. Male rats with SFO lesions or sham lesions were given a 10 mg/kg dose of furosemide sc followed by either 2- or 10-ml/kg loads of tap water 2, 4 and 18 hr later (6 or 30 ml/kg total load) in different groups. No other food or fluids were available. Two hours after the last load, the rats were given access to water and 0.3 M NaCl for 2 hr. The results (below) showed that: (1) Water intake during the test depended on the lesion condition; and (2) saline intake depended on the amount of water loaded during the depletion. Thus, SFO-lesioned rats can express a robust salt appetite after sodium depletion if they are hydrated equivalently to control rats. This supports our view that the SFO is not necessary for the expression of ANG II-induced salt appetite.

	Lesion treatment/water load			
Intake	sham/30	sham/6	SFOX/30	SFOX/6
Water	8.0±1.3	6.2±1.8	1.5±0.6*	2.5±0.6*
Saline	10.8±1.6	8.6±1.5*	10.7±1.7	6.1±0.9*
n	10	8	7	10

*main effect, $p<.05$ (mean±SE). Supported by NS22274.

660.15

AREA POSTREMA MODULATION OF FLUID INTAKE AND NEUROHYPOPHYSEAL HORMONE SECRETION AFTER HYPERTONIC NaCl ADMINISTRATION. K.S. Curtis*, J.G. Verbalis, and E.M. Stricker. Depts. of Neuroscience and Medicine, U. of Pittsburgh, Pittsburgh, PA 15260

The area postrema is a caudal brainstem circumventricular organ thought to be involved in body fluid homeostasis. Rats with area postrema lesions (APX) show enhanced spontaneous salt intakes and reduced urinary excretion of salt loads. The present studies examined drinking and neurohypophyseal responses to systemic administration of hypertonic saline (HS) in rats with APX. When water was available after HS (2 ml 2 M NaCl, iv), intake by rats with APX (10.4 ± 0.8 ml; n=7) was greater than that by control rats (6.6 ± 0.8 ml; n=17) during a 30-min drinking test, while excretion of the salt load was reduced. In contrast, when only 0.5 M NaCl was available, the enhanced NaCl intake by rats with APX (n=6) was unaffected by iv HS in a 30-min drinking test (7.5 ± 0.7 ml). Baseline plasma oxytocin levels (pOT) in rats with APX (4.4 ± 0.8 pg/ml; n=6) were not different from those in control rats (3.1 ± 0.9 pg/ml; n=11). Although iv administration of HS stimulated pituitary release of oxytocin in both groups, pOT in rats with APX were blunted 30 min after HS (13.5 ± 1.6 pg/ml) compared to those in control rats (21.4 ± 3.2 pg/ml). These results suggest some anatomical segregation of HS-induced drinking and neurohypophyseal responses, and are consistent with our previous proposal that the robust spontaneous salt intake of rats with APX may reflect reduced inhibition by central oxytocin neurons.

660.16

MINERALOCORTICOID INDUCED SODIUM APPETITE IN GROUP HOUSED MALE MICE. **K.K. Henricks*, Z.A. Rodd, B.C. Dudek, & N.B. McCutcheon. Dept. of Psychology, University at Albany: SUNY, Albany, NY 12222.**

Up to now, research investigating sodium hunger in mice has failed to produce evidence that mineralocorticoids are involved in sodium appetite. In our own laboratory, doses of deoxycorticosterone acetate (DOCA) ranging from 1mg/kg to 20mg/kg have failed to induce a sodium appetite. However, recent research with fludrocortisone suggests that the mouse, like the rat, does possess mineralocorticoid reactivity for sodium appetite, but, unlike the rat, appears to have a strong dependency on an accompanying glucocorticoid action. Previous experiments were conducted with singly housed, socially isolated mice, a condition which may have altered neural modulating factors that interact with mineralocorticoid control of sodium appetite. Two such factors associated with housing condition and sodium appetite control are corticosterone and serotonin. Therefore, the present experiment was conducted in order to study the effect of DOCA on sodium appetite in mice under housing conditions that attempted to eliminate the reduction of corticosterone and the alteration of the serotonergic system associated with social isolation. Male GHSC mice were group housed and given two counterbalanced treatment conditions - a single daily injection of either 10 mg/kg DOCA or oil vehicle for two consecutive days. Group housed male GHSC mice drank, over 24-h of continuous access to water and 3% NaCl, a significantly larger amount of NaCl after injections of DOCA than after injections of the vehicle. This degree of DOCA effect in mice is comparable, on a ml/kg basis, to that reported in rats with high dose DOCA injections.

660.17

DISSECTION OF MEAL CONTROLS IN COLITIS-INDUCED ANOREXIA. E.D. Kustra*, S.Amarelo, S.M. Collins and H.P. Weingarten. Dept. of Psychology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

Acute inflammation of the colon (ie, colitis) results in a large but transient anorexia of approximately 3 days duration. Studies indicate that the anorexia is caused by a specific reduction of meal size, not meal frequency, leading to the hypothesis that the suppression of eating reflects an exaggerated response to cues that normally signal meal termination. In order to obtain the greatest stimulus control over meals, we compared meal sizes in colitic and control rats using the intraoral feeding preparation. Male Sprague-Dawley rats were maintained on powder chow and habituated to one intraoral feeding session per day using liquid diet. Then, colitis was induced by infusing 25 mg trinitrobenzenesulfonic acid (TNB) in .25 ml 50% ethanol directly into the colon (N=11). Control rats (N=10) received equivolume infusions of 50% ethanol. Meal sizes were approximately 2.5 times larger when intraoral, compared to real, feeding. TNB-treated rats demonstrated a much attenuated and smaller inhibition of intake on these intraoral meals even though they displayed the expected pattern of anorexia in home cage intake. In a second study, we specifically compared the satiety response of TNB and control rats to intragastric loads. Rats were adapted to a 4 hr/day feeding schedule. Then, half the rats were treated with TNB; the other half with the ethanol vehicle. On Days 1-5 post-treatment, half of the animals in each treatment group received a 5 ml liquid diet preload 20 min prior to the 4 hr feeding period. The other half received no preload. TNB-treated rats displayed the expected pattern of anorexia across days. However, the 5 ml preload had no differential effect on the amount eaten by TNB or control groups. These data suggest that some aspect of the preparatory feeding responses that are eliminated by intraoral feeding may be necessary for the complete expression of TNB-induced anorexia and that differences in postingestive satiety alone may be insufficient to explain fully the small meals associated with colitis. Collectively, these observations suggest that elucidation of the mechanisms accounting for the anorexia of colitic rats will not be simple.

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660.18

THE USE OF MULTIDIMENSIONAL SCALING IN THE APPRAISAL OF THE RELATIONSHIP BETWEEN BRAINSTEM ELECTRODE PLACEMENT, WEIGHT GAIN, AND FOOD INTAKE. J. Stenger*, S. Jordan and C. Bielajew. School of Psychology, Univ. of Ottawa, Ottawa, Ont., K1N 6N5, Canada.

A multidimensional scaling analysis was used to examine inter-element proximity data from rats that had received chronic electrical stimulation to the brainstem region. The variables were the three histological planes, weight gain, and food intake. The results of the principle components analysis yielded four factors, two of which, weight gain and food intake, were combined into one (mass) factor. The proximity matrix was generated for 45 rats based on the histological data and the factorial scores from the principle components analysis. The multidimensional scaling analysis yielded an $r^2=0.938$ and a stress value of 0.118. Visual inspection of the two dimensional stimulus map revealed that cases formed two clusters on one dimension. The separation along this dimension was influenced by weight gain, food intake, and electrode placement. Not surprisingly, one cluster largely consisted of rats with ventromedial hypothalamic electrode placements, whereas the second cluster contained animals with extra-VMH placements. It appears that weight gain and food intake also differs for these two groups; this theory is born out by previous work from this laboratory.

660.19

A RETROSPECTIVE STUDY OF THE EFFECTS OF CHRONIC SMOKING ON REGIONAL CEREBRAL BLOOD FLOW DURING COGNITIVE PROCESSING. J.L. Austin-Lane*, B.S. Kirkby, J.D. Van Horn, G. Esposito, D.R. Weinberger, K.F. Berman. Unit on PET, Clinical Brain Disorders Branch, NIMH, Bethesda, MD 20892-1384.

Previous studies on nicotine and regional cerebral blood flow (rCBF) have focused on acute effects of cigarette smoking, but very little work on chronic effects of smoking has been done. Using the oxygen-15 water positron emission tomography (PET) method, we retrospectively examined global and regional CBF in 10 chronic smokers (mean age 31.2+9.6) and 10 subjects who had never smoked (mean age 29.9+7.8) while they performed the Wisconsin Card Sorting Test and a sensorimotor control task. Subjects had no caffeine or nicotine for four hours prior to the study, and all subjects were right-handed. Absolute rCBF was calculated on a pixel-by-pixel basis with a least squares method. Data were normalized, pixel-by-pixel, as a ratio of the global mean for regional analyses and regions of interest were drawn on co-planar MRIs for each subject. There was a trend (t=-2.09, p=.052) for the smokers to have higher global CBF (51.3+8.58 ml/100g/min) than the non-smokers (44.6+5.41 ml/100g/min), while their normalized rCBF in the left superior frontal gyrus was lower during both task (t=3.58, p=.002) and control (t=2.54, p=.02). For the smokers, Spearman rank order correlations between smoking index (#cigarettes per day x #years) and rCBF revealed a negative relationship between smoking history and rCBF in the left superior temporal gyrus during the control task (r=-.77, p=.009). These data, although preliminary, suggest that effects of chronic smoking on brain activity can be seen, even in the absence of acute nicotine effects. These results should be interpreted with caution since this study has a small sample size and is retrospective instead of prospective in nature, and many comparisons were made.

STRESS: PRECLINICAL AND CLINICAL STUDIES

661.1

STRESS AND PENTYLENETETRAZOL INCREASE THE RELEASE OF DOPAMINE IN THE PREFRONTAL CORTEX OF ROMAN HIGH-AVOIDANCE (RHA) BUT NOT LOW-AVOIDANCE (RLA) RATS. O. Giorgi, D. Lecca, G. Carboni, V. Frau, V. Valentini, A. Fernandez*[1], G. Di Chiara and M.G. Corda. Dept. Toxicol., Univ. of Cagliari, Italy and [1]Dept. Pharmacol. and Psych., Autonomous Univ. of Barcelona, Spain.

RHA and RLA rats are selected and bred for rapid versus poor acquisition of two-way avoidance behavior in a shuttle box. They also show many other behavioral differences related to emotional factors, RLA rats being emotionally more sensitive. To delineate further the functional factors underlying the different emotivity levels of RHA and RLA rats, we used brain microdialysis to compare the effects of tail pinch (TP, 40 min) and subconvulsant, anxiogenic doses of pentylenetetrazol (PTZ, 15 mg/kg, i.p.) on the release of dopamine (DA) in the prefrontal cortex (PFCX) and nucleus accumbens (NA) in both rat lines. The basal release of DA (fmol/20 µl) was higher in the PFCX of RHA rats (12.6 ± 0.7) than in RLA rats (8.1 ± 0.4, $n = 21$, $p < 0.05$) but no line-related differences were observed in the basal DA release in the NA (RHA: 73 ± 4, RLA: 67 ± 3). TP caused a significant increase in DA release in the PFCX of RHA rats (net release above basal value in 2 h after beginning of TP, fmol: 10.9 ± 1.9, $p < 0.05$) but not in RLA rats (net release: 3.2 ± 2.6, n.s.). Similarly, PTZ increased significantly DA release only in the PFCX of RHA rats (net release, fmol/2 h after PTZ: RHA, 43 ± 7, $p < 0.05$; RLA, 8 ± 5, n.s.). In contrast, TP and PTZ failed to affect DA release in the NA of either line. It is proposed that the activation of the mesocorticolimbic DAergic pathway induced by TP and anxiogenic doses of PTZ may reflect an increased attention of the animal and/or the activation of cognitive mechanisms in an attempt to actively cope with the stressor. These mechanisms appear to be lacking in RLA rats, which are "passive" copers.

661.2

DIFFERENTIAL EFFECTS OF RESTRAINT, IMMERSION AND ELECTRIC FOOT SHOCKS. S. Retana-Marquez, A. Ferreira Nuño, E. Dominguez Salazar and J. Velazquez-Moctezuma*. Lab. of Psychobiology, Universidad Autonoma Metropolitana-Iztapalapa, Mexico city, C.P. 09340.

Previous results have shown that stress response depends on the nature of the stressor. To further analyze this notion, male rats were submitted either to immobilization during 2 (IMB2) hrs, immobilization during 6 (IMB6) hrs, electric foot shocks (EFS) during 5 mins or immersion in cold water (WIM) during 15 min, for 1 day or daily during 15 and 20 days. To assess stress effect, male sexual behavior was evaluated when the stress period had ended, thereafter blood was collected to determine corticosterone titers. Spleen, thymus, liver, testicles, adrenal and seminal glands were also obtained. WIM decreased sexual behavior performance during all periods tested. EFS deteriorated sexual behavior only in days 15 and 20. IMB2 had no effect and IMB6 stimulated sexual performance. All the stressors produced significant increases in costicosterone titers during days 15 and 20, but not in the first day. WIM induced the higher elevation, whereas EFS elicited only slight increases. Only IMB6 induced noticeable changes in total weight of organs. Spleen increased its weight after 20 days of stress and testicles increased their weight after 15 days of stress. This results support the notion that the stress response will depend on the characteristics of the stressful situation.

661.3

STRESS-INDUCED HYPERTHERMIA IN INDIVIDUALLY HOUSED MICE. J.A.M. van der Heyden *, T.J.J. Zethof and B. Olivier Dept. Pharmacology, Solvay Duphar BV, PO Box 900, 1380 DA Weesp, The Netherlands

Measurement of stress-induced hyperthermia (SIH) in group-housed mice requires a large number of animals and is time-consuming. We adapted this method for use in individually housed mice. The effect of various stressors on the rectal temperature of individually housed mice was tested. Repeated temperature measurement at a 10 min interval was chosen as stressor since this enabled measurement of basal temperature and hyperthermic response in each animal. The maximal hyperthermia is reached after 30 min, but 70 % of the response is reached after 10 min. Prior injection of the animals also results in a modest hyperthermia, that is no longer observed if the animals are treated 60 min prior to the first temperature measurement. Various benzodiazepines (diazepam, oxazepam, alpidem) dose-dependently suppressed the stress-induced hyperthermia. Similar effects were found for alcohol and the 5-HT_{1A} agonists buspirone, ipsapirone, flesinoxan and 8-OH-DPAT. Of the various 5-HT drugs tested, TFMPP and eltoprazine were active in this model. No effect was found after administration of ondansetron, ketanserin, fenfluramine, DOI or m-CPP. Of the antidepressant drugs tested, only mianserine showed an anxiolytic effect, but not imipramine, amitriptyline, chlorimipramine and fluoxetine. At high doses also the neuroleptic drugs haloperidol and chlorpromazine attenuated the stress-induced hyperthermia. No effects were found after administration of the glutamate antagonist MK 801 and the CCK_B antagonist CI-988.

In conclusion, the stress-induced hyperthermia model in individually housed mice is a fast and reproducible screening test for anxiolytic activity. The major advantages of this model compared to that described in group-housed mice are the reduction in time and animals needed for an experiment.

661.4

EFFECTS OF CHLORDIAZEPOXIDE ON SOCIAL SEPARATION STRESS IN YOUNG DOMESTIC FOWL; A NOVEL MODEL FOR SCREENING ANXIOLYTIC DRUG EFFICACY. G. S. Watson, M. J. Mark, E. S. Carlson and K. J. Sufka* Departments of Psychology and Pharmacology, University of Mississippi, Oxford, MS 38677.

In response to concerns over issues of validity and utility of traditional procedures for screening anxiolytic drugs, the present study sought to determine whether a relatively inexpensive social separation stress biobehavioral assay, possessing both face and construct validity, would demonstrate predictive validity by screening for the anxiolytic effects of the benzodiazepine agonist chlordiazepoxide (CDP). Thirty min after IM injections of CDP (Exp. 1: 0.0, 3.0, and 10.0 mg/kg; Exp. 2: 0.0, 1.0, & 3.0 mg/kg), eight-day-old cockerels were placed into observation chambers in isolation or with two social companions for a formalin (0.10%) nociceptive test. At the highest dose, CDP produced a sedative effect, independent of the stress manipulation, as indexed by a decrease in ventral recumbency latency. Isolated chicks exhibited distress vocalizations; CDP reversed this separation stress effect in a dose-dependent fashion. Isolated chicks displayed hypoalgesia on the formalin test. CDP (1.0 and 3.0 mg/kg) attenuated this separation stress hypoalgesic effect. Chicks treated with 10 mg/kg CDP exhibited few pain-related behaviors. However, this specious hypoalgesic effect was likely due to CDP's potent sedative effects at this dose. Finally, CDP did not affect respiration rate, but did produce a dose-dependent hypothermic effect. The demonstration of this paradigm's predictive validity suggests that the chick social separation stress model may serve as a useful assay for screening anxiolytic drug effects.

661.5

SEXUALLY DIMORPHIC EFFECTS OF NEONATAL ENVIRONMENTAL DISRUPTIONS ON DEPRESSIVE BEHAVIOR AND PITUITARY-ADRENAL HORMONE FUNCTIONING OF THE ADULT RAT E.P. Zorrilla*, S. Shah, J. Kessler, H. Lieberman, E. Redei. Depts.of Psychology and Pharmacology, U.Pennsylvania, Philadelphia, PA 19104.

Many studies have documented changes in emotional behavior and functioning of the hypothalamic-pituitary-adrenal axis in adult male rats subjected to handling or maternal separation as neonates. However, many of these did not control adequately for litter effects (e.g., genetic differences in parents, gestational/rearing differences), nor did they test for sex differences in their effects. Therefore, we subjected 40 litters derived from Sprague-Dawley dams mated in our colony, to 1 of 5 postnatal conditions: cage-cleaning control (C), daily, 1-hour maternal/litter separations through the 1st or 2nd postnatal week (S1,S2), or a daily handling control for the separation groups (H1,H2). At 60 days of age, analyses of pups' (n=457) behavior in the Porsolt forced swim test (FST) and HPA-responses were performed at the unit of litter, rather than at the unit of pup. Females exhibited more immobility in the FST than males ($p<.0005$). This difference was reduced by both early separation and handling (Sex X Treatment, $p<.005$), as both S and, especially, H males, showed female C levels of 'behavioral despair.' Only H females showed significantly more immobility than C females ($p<.05$). Furthermore, week 2 females, but not males, showed greater increases in immobility than their week 1 counterparts ($p<.05$). Sexually dimorphic effects of the treatments also were observed on basal levels of corticosterone (H=S>C in females, but not males) and ACTH (H=S<C in males, H1>H2 and S2>S1 in females). The present study provides further evidence that adult affective behavior can be altered by early life experiences, reinforces the need to address litter effects, and suggests that some imprinting effects are maternally-mediated.

661.6

STRESSOR EXPOSURE PRODUCES LASTING EFFECTS ON SLEEP/WAKING STATE RELATED CELL ACTIVATION OF THE CAT PARAVENTRICULAR HYPOTHALAMUS. M.P. Kristensen*, D.M. Rector, G.R. Poe and R.M. Harper. Interdepartmental Program in Neuroscience and Department of Anatomy and Cell Biology, UCLA School of Medicine, Los Angeles, CA, 90095.

The paraventricular hypothalamus (PVH) mediates hypothalamo-pituitary-adrenocortical (HPA) axis activation and is subject to multi-level feedback-regulation. Stressors increase PVH discharge acutely, but stress, particularly when repeated or prolonged, can introduce enduring modifications of neural function.

Our purpose was to examine whether PVH neuronal responsivity to perturbations by endogenous, non-stressors can be influenced by stress. We assessed whether PVH activity patterns characteristic of specific sleep/waking states were altered by an auditory stressor. Activity changes were indexed by quantifying tissue light reflectivity, which is inversely proportional to cell discharge.

A coherent image conduit, directly coupled to a CCD camera, was stereotaxically positioned in the PVH of 4 cats. Electrodes were placed for measuring neck EMG, EOG, and cortical EEG to monitor behavioral state. After surgical recovery, multiple video-recordings of scattered light were obtained from freely behaving cats during spontaneously varying sleep/waking states before and after 5 minute exposure to an auditory stressor (white noise > 90 dB). Video-images were grouped by state, and normalized overall, as well as subarea, activity changes were calculated.

Prior to stress, overall PVH activity increased during quiet and active sleep compared to waking states. Activity levels did not change significantly between states after stress, though a blunted variation similar to control patterns was apparent. Activity changes accompanying awakening from quiet or active sleep were reduced by 2/3 after noise exposure.

The results demonstrate state-specific PVH activity patterns, and suggest lasting effects of stress at the cellular level on PVH function. We speculate that a blunted response of state-changes after stress may arise as a result of altered state control and/or HPA feedback effects on the PVH.

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661.7

ENHANCEMENT OF HIPPOCAMPAL PRIMED BURST (PB) POTENTIATION BY DEHYDROEPIANDROSTERONE SULFATE (DHEAS). D.M. Diamond[1]*, B.J. Branch[1], M. Fleshner[2], and G.M. Rose[1]. Dept. of Pharmacology, Univ. of Colorado Health Sci. Ctr. and VAMC[1], Dept. of Psychology, Univ. of Colo. Boulder[2].

The neurosteroid DHEAS is produced in the brain and is the major secretory product of the human adrenal cortex. Functionally, DHEAS is described as an anti-glucocorticoid (*Mol. Cell. Biochem.*, 131:99, 1994). However, little is known concerning the effects of DHEAS on hippocampal function. We have provided the first analysis of the effects of DHEAS on hippocampal primed burst (PB) potentiation, an electrophysiological model of memory.

Male rats were administered DHEAS (12-96 mg/kg, sc), then anesthetized with urethane, and the CA1 region of the hippocampus was electrically stimulated (see *Hippocampus*, 2:421-430, 1992). ***There was an inverted-U relationship between the dose of DHEAS and PB in non-stressed rats: whereas 24 and 48 mg/kg enhanced PB, 12 and 96 mg/kg were without effect. Further study revealed that: 1) DHEAS (24 mg/kg) was effective at enhancing PB when given before the rats were stressed; and 2) DHEAS (24 mg/kg) was ineffective at enhancing PB when given after the rats were stressed.*** These findings indicate that DHEAS could protect the hippocampus against the inhibitory effects of stress on synaptic plasticity, but only when the neurosteroid was administered in advance of the stressful event.

661.8

METYRAPONE-INDUCED ENHANCEMENT OF HIPPOCAMPAL PB POTENTIATION CORRELATES WITH TYPE I RECEPTOR BINDING, BUT NOT WITH PERIPHERAL CORTICOSTERONE. B.J. Branch[1]*, M. Fleshner[2], M.J. Meaney[3] and D.M. Diamond[1]. Dept. of Pharmacology, Univ. of Colorado Health Sci. Ctr. and VA Medical Center[1], Dept. of Psychology, Univ. of Colorado, Boulder[2], Douglas Hosp. Res. Ctr., Montreal, Quebec[3].

Corticosterone modulates electrophysiological models of memory, including long-term (LTP) and primed burst (PB) potentiation. We have tested the hypothesis that a dose-dependent reduction of endogenous corticosterone by metyrapone would correlate with the magnitude of PB. Rats were given metyrapone (25, 50, 100 or 200 mg/kg, sc) prior to being anesthetized with urethane, and the hippocampus was electrically stimulated (*Hippocampus*, 2:421-430, 1992). ***All 4 doses of metyrapone were equally effective at reducing serum corticosterone levels to 10-15 μg/dl; however, only the 50 mg/kg dose enhanced PB and reduced the number of available hippocampal Type I receptors.*** Also, doses ≥ 50 mg/kg increased available Type II receptors, independent of serum corticosterone levels. Corticosterone (20 mg/kg, sc) blocked metyrapone effects on PB and Type I binding. This work suggests that metyrapone can affect hippocampal plasticity and glucocorticoid receptors by means other than a reduction in serum corticosterone levels, possibly via its capacity to increase levels of ACTH, DHEA and deoxycorticosterone.

661.9

PSYCHOLOGICAL DISTRESS IN HIV-1 DISEASE: RELATIONSHIP TO HYPOCHOLESTEROLEMIA. G. Shor-Posner*, D. Feaster, T. Baldewicz, N. T. Blaney, M. Miguez-Burbano, K. Goodkin, C. Eisdorfer, M.K. Baum. Departments of Epidemiology and Psychiatry, University of Miami School of Medicine, Miami, Florida.

Disturbances in serum lipid concentrations have been associated with behavioral abnormalities, possibly mediated by serotonin pathways that are altered in HIV-1 infected individuals. The present study evaluated serum cholesterol levels and psychosocial status in 118 HIV-1 seropositive (CDC Stages II, III n= 96; CDC Stage IV 2A n=22) and 52 seronegative homosexual men aged 20-55. Levels of cholesterol were obtained from serum extracts after reaction with Lieberman-Buchard color reagent. Psychological distress was measured with the total mood disturbance score from the Profile of Mood States. Hypocholesterolemia (<150 mg/dl) was significantly related to increased distress ($p=.03$) in both the seropositives and seronegatives. As compared to distress levels of 20.73 ± 31.42 in the seronegatives, distress tended to be higher in the seropositive men (32.81 ± 32.97, $p<0.06$), who had widespread hypocholesterolemia (41% vs 25% in seronegatives, $p<0.05$). Clinical distress (score >60) occurred more frequently among those with hypocholesterolemia (26%), relative to 13% with normal range cholesterol levels ($p=0.03$). These findings demonstrate an important influence of lipid status on psychological function.

661.10

STARTLE AND NEUROENDOCRINE RESPONSES IN SHY CHILDREN L.A.Schmidt, N.A. Fox, E.M. Sternberg, P.W. Gold, C.C. Smith and J.Schulkin* Inst. for Child Study, Univ. of Maryland, College Park, MD 20742.

The relations among behavioral inhibition, morning salivary cortisol, and the acoustic startle response in a sample of 4-year-old children were examined. Analyses revealed a significant relation between morning cortisol level and acoustic startle amplitude. Children who had elevated morning cortisol levels exhibited an augmented startle response. Analyses also revealed a significant relation between 14-month behavioral inhibition and morning cortisol at 4-years. Children who displayed fear and wariness in response to novel stimuli at 14-months of age had elevated morning cortisol at 4-years. These findings are consistent with recent studies of fear responses in animals in which CORT can potentiate fear, and CRH expression in the central nucleus of the amygdala, and are consistent with work on neuroendocrine responses in fearful children. High levels of cortisol in inhibited children may induce CRH in the central nucleus of the amygdala, exacerbating their fearfulness.

661.11

HUMAN RESPONSE TO UNCONTROLLABLE STRESS AND HIGH DOSE NALOXONE J. Fertig*, R. Peters, J. Leu. Walter Reed Army Institute of Research, Washington, D.C. 20307

Forty healthy male subjects were exposed to bursts of 95 dB noise while attempting to solve a visual-spatial task under either controllable stress (CS) or uncontrollable stress (UCS) conditions. CS subjects could terminate both stressors while their yoked UCS partners could not. Measures of physiologic reactivity, biochemical response and mood were monitored throughout the study. Stress induction was followed by a double blind infusion of the opiate antagonist naloxone (1.5 mg/kg) or placebo. A muscle ischemia pain tolerance test was given following drug administration. Physiologic response and several biochemical markers of the stress response were differentially elevated in the two conditions. UCS subjects also reported increased helplessness, lack of control and total mood disturbance. Naloxone administration augmented the stress induced rises in cortisol, heart rate, negative mood and physical symptoms but did not affect diastolic or systolic blood pressure. In addition, naloxone had a differential effect on pain tolerance depending upon subject's prior exposure to CS or UCS. Pain intensity ratings were highest for CS exposed subjects and lowest for UCS subjects. Results suggest that lack of control over psychological stressors can produce differential responses in physiological, biochemical and behavioral systems.

661.12

CORTISOL RESPONSE AND MEMORY FUNCTION FOLLOWING A PSYCHOLOGICAL STRESS IN HEALTHY ELDERLIES : RELATIONSHIP WITH PAST CORTISOL HISTORY.

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Saliva cortisol responses to the psychological stress of public speaking was investigated in 18 healthy elderly subjects previously shown to present different patterns of cortisol secretion over a period of 3 to 6 years. A test of explicit (cued recall) and implicit (word-stem completion) memory was given to subjects before and after a non-stressfull condition (attention task) and before and after the stressful condition. Correlational analyses performed between memory scores and cortisol responses showed that the explicit recall during the non-stressful condition was negatively correlated with post-recall cortisol levels (r=-0.72) and not with pre-recall cortisol levels (r=-0.24). This correlation lasted for 10 minutes and disappeared for the next cortisol sample. No correlation coefficient reached significance level for the implicit memory task. The explicit recall during the stressful condition was significantly and negatively correlated with both pre-recall/post-stress cortisol levels (r=-0.59) and post-recall/post-stress cortisol levels (r=-0.52), showing that the subjects who reacted to the stress with increases in cortisol levels presented the greater memory impairment as tested after the stressor. This correlation lasted 21 minutes and disappeared for the next cortisol sample. No correlation coefficient reached significance level for the implicit memory task. Finally, the cortisol slope of subjects as measured over a period of 3 to 6 years was negatively correlated with changes in explicit and implicit memory performance after the stressful condition (r=-0.33 & -0.31) but not with explicit and implicit performance after the non-stressful condition (r=-0.11 & 0.09). These results show that aged subjects with a history of increasing cortisol levels over the years present a stronger cortisol response to a psychological stressor and a greater memory decline after a stress than aged subjects showing moderate increase or decrease in cortisol levels with years.

661.13

STRESSING THE STRESS CONCEPT: QUANTITY MAKES QUALITY

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Contrary to the Selye's concept of stress as a basic hormonal mechanism of adaptation, this physiological phenomenon is often considered in medicine, and presented through the media as a ravaging event resulting, directly or indirectly, in various diseases. This conceptual distortion originates from the well documented deleterious effects of high doses of glucocorticoid hormone, or of chronic fierce stress exposure. On these bases it has been postulated that a high glucocorticoid milieu can damage the hippocampus and exacerbate neurotoxic lesions. We have found that the hippocampal injury by kainic acid is accrued neither by aging nor by repeated stress exposures, two conditions accompanied by or causing large, albeit physiological, increases in plasma glucocorticoid level. A potentiation of the neurotoxic action was encountered only in some strains of rats following injection of corticosterone in doses producing in the periphery and in the brain concentrations of the hormone a hundred times greater than normal. The brain glucocorticoid receptor (GR) binding capacity is controlled by neurotransmitters: dysfunction of the hypothalamo-pituitary-adrenocortical axis during depression, as well of the negative feedback function of the GR, and the relief by antidepressants are operated through loss and reinstatement, respectively, of neurotransmitter action. A totally different image emerges when physiological increases of the hormone are considered. For instance, we have found that rats lactated by a mother with a moderate hypercorticosteronemia (of the same degree as following low-intensity stress exposure), in adulthood show "better" adaptive behaviors and a reduced stress responsiveness. In conclusion, in spite of the principle that quality is more fundamental than quantity, in stress related phenomena quantity appears more momentous than quality. (Supported by MURST 1994 and CNR 94.00479.PF 40).

661.14

BRAIN CHEMISTRY OF LEARNED HELPLESSNESS: NEURAL NETWORK FOR DEPRESSION MODEL. F. Petty* and G. Kramer. Department of Veterans Affairs Medical Center and Department of Psychiatry, University of Texas Southwestern Medical School, Dallas, Texas 75216.

For many years, the biochemical theories of mood disorders featured one or another of the biogenic amines as key players in the development of depressive symptoms, and in their amelioration by pharmacological treatments. By default, these monoamine theories focused on locus ceruleus or dorsal raphe as anatomical loci for biochemical changes. We have developed a new, multi-transmitter, multi-brain region model for learned helplessness which incorporates the traditional biogenic amine theories, and also explicates the roles of the amino acid neurotransmitters including GABA, glutamate, and glycine. This neuronal model incorporates regions of the limbic system known to regulate mood, and accounts for complex neurotransmitter interactions, particularly in the medial prefrontal cortex, where the cascade of events leading from inescapable stress to behavioral depression begins. Other brain regions considered in the model include entorhinal cortex, hippocampus, lateral septum, hypothalamus, amygdala, and nucleus accumbens. This model will be compared to the neuronal maps being developed for clinical depression using brain imaging. Additionally, new data from *in vivo* microdialysis experiments in progress will be presented.

MONOAMINES AND BEHAVIOR: DOPAMINE I

662.1

EFFECTS OF SELECTIVE AND NON-SELECTIVE DOPAMINE ANTAGONIST ADMINISTRATION IN PREFRONTAL CORTEX ON DRL RESPONDING IN THE RAT. J.D. Sokolowski* and J.D. Salamone. Dept. of Psychlogy, University of Connecticut, Storrs, CT 06269-1020 USA

Previous research utilizing dopamine depletions in the medial prefrontal cortex of the rat demonstrated substantial deficits in operant responding on the differential-reinforcement-of-low-rates 30 sec schedule. In the present experiments, rats were locally injected with one of four dopamine antagonists: the non-selective drug cis-flupenthixol, the moderately D2 selective drug haloperidol, the highly D1 selective antagonist SCH23390, the highly D2 selective antagonist sulpiride. Doses of 5-20 nanomoles per side were injected bilaterally in 1.0 ul total volume, and control injections consisted of lactic acid vehicle. Local injections of flupenthixol increased total number of responses, with the most effective dose being 10 nanomoles. Analysis of interresponse times demonstrated that, at the 10 nanomole dose, there was a 3-4 fold increase in interresponse times in the range of 0-1.0 sec. This indicates that much of the increase in responding produced by flupenthixol was in the form of increased "bursts" of responses that closely followed one another. Local injections of haloperidol in the prefrontal cortex produced effects similar to those observed with flupenthixol. However, preliminary data indicate that the effects of intra-prefrontal SCH23390 and sulpiride were less robust than the effects of flupenthixol or haloperidol in the dose range tested.

662.2

PREFRONTAL CORTICAL GABA RECEPTORS MODULATE STRESS-INDUCED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS. M. D. Doherty* and A. Gratton, McGill Univ., Douglas Hosp. Res. Ctr., Montreal, Canada, H4H 1R3.

We have recently reported that local D1-, but not D2-, receptor blockade in medial prefrontal cortex (PFC) potentiates stress-induced dopamine (DA) release in nucleus accumbens (NAcc). This finding is consistent with several other lines of evidence indicating that meso-PFC DA neurons exert an indirect inhibitory influence on the NAcc DA response to various stimuli including stress. The PFC also contains GABA interneurons and these too may be involved in modulating the DA stress response in NAcc. We examined this possibility by monitoring restraint-induced increases in NAcc DA-dependent electrochemical signals following bilateral intra-PFC injections (1 nmole/side) of GABA-A and GABA-B agonists (muscimol and baclofen) and antagonists (bicuculline and phaclofen). Intra-PFC baclofen significantly attenuated the peak amplitude and the duration of the NAcc DA response to stress; the GABA-A agonist muscimol produced a similar but less potent and non-significant inhibitory effect. In contrast, PFC GABA-A receptor blockade with bicuculline elicited seizures, whereas the GABA-B blocker phaclofen had no effect. However, intra-PFC phaclofen was found to potentiate the NAcc DA response to stress when it was co-injected with the D2 receptor antagonist sulpiride at a dose we have shown previously to have no effect (Doherty & Gratton, 1994). These findings indicate that PFC projection neurons that modulate the DA stress response in NAcc are regulated in part by GABA acting primarily, but not exclusively at the GABA-B site. They also suggest that, unlike D1 receptors, an involvement of PFC D2 receptors in modulating the NAcc DA response to stress may depend on the activity of cortical GABA interneurons. *Supported by the Medical Research Council of Canada.*

662.3

CHANGES IN NUCLEUS ACCUMBENS DOPAMINE EFFLUX ELICITED BY SCHEDULED AND UNSCHEDULED FOOD PRESENTATION. N.R. Richardson* and A. Gratton, McGill Univ., Douglas Hosp. Res. Ctr., Montreal, Canada, H4H 1R3.

We have recently reported evidence that dopamine (DA) release in nucleus accumbens (NAcc) increases during the moments leading to an operant response (lever-press) reinforced with food (condensed milk) and that consumption of the earned reward is associated with a cessation or inhibition of NAcc DA transmission (Richardson & Gratton, 1994). These and similar other findings are generally consistent with the idea that NAcc DA transmission increases in response to the incentive, behavioral activating properties of rewards. In order to test this idea further, we used voltammetry to monitor the changes in NAcc DA efflux associated with non-contingent presentations of a 0.2 ml meal of condensed milk delivered over 30 sec. Animals received meals either at fixed intervals of 52 secs (FI 52) or on a variable interval (VI 52) schedule of 32,35,40,45,53,64 and 95 secs. Meals presented on the FI schedule were preceded by pronounced signal increases that peaked within a few secs of receiving the anticipated milk reward before decreasing as the animals consumed the milk. These biphasic changes were remarkbly similar to those we have observed previously in animals lever-pressing for food. Similar decreases in signal were observed when animals consumed meals presented on the VI schedule. Under this condition, however, each meal was followed by an increase in signal that peaked rapidly and remained elevated until the next milk presentation. These findings reinforce the idea that DA transmission in NAcc increases in anticipation of a positive outcome. They also suggest that such anticipatory elevations in meso-NAcc DA activity increase as a function of reducing the predictability of the outcome. *Supported by the Medical Research Council of Canada.*

662.4

DOPAMINE AND PLAY BEHAVIOR IN JUVENILE RATS: RELATIVE INVOLVEMENT OF D2 AND D3 RECEPTORS. S.M. Siviy* and A.L. Milburn. Department of Psychology, Gettysburg College, Gettysburg, PA 17325.

Previous work from this lab has suggested that dopamine might be involved in the normal elaboration of playfulness among juvenile rats. Specifically, low doses of the D2/D3 agonist quinpirole tend to increase pinning, an index of play in the rat, while higher doses reduce pinning. Since quinpirole acts at both D2 and D3 receptors, it is not clear from these data which receptor subtype might be responsible for these effects. In the present study, the effects of quinelorane, a more selective D2 agonist, and 7-OH-DPAT, a selective D3 agonist, on play were assessed. Male rats (25-35 days old) were housed individually and given daily 5 minute opportunities to play. One group of rats were injected SC with either vehicle or one of four doses of quinelorane (0.003, 0.01, 0.03, 0.1 mg/kg) 30 minutes prior to a play session. Another group of rats were injected SC with either vehicle or one of four doses of 7-OH-DPAT (0.003, 0.01, 0.03, 0.1). The frequency of pinning was recorded and play bouts were also videotaped for later analysis. 7-OH-DPAT had no significant effect on pinning at any of the doses. Quinelorane significantly increased pinning at the lowest dose (0.003 mg/kg) and decreased pinning at the higher doses, with play being completely abolished at 0.1 mg/kg. These data suggest that dopamine, acting at D2 receptors, can have biphasic effects on play. On the other hand, D3 receptors, which are localized preferentially in limbic structures, do not appear to be involved in play behavior.

662.5

CHOLERA TOXIN INFUSION INTO NUCLEUS ACCUMBENS: INTERACTIONS WITH MOTOR ACTIVITY, DOPAMINE AGONISTS, AND CONDITIONED REINFORCEMENT. A.E. Kelley,* M. Holahan, M. Finn. Dept. of Psychiatry, Univ. of Wisconsin Medical School, Madison, WI 53706

Cholera toxin (CTX) is known to cause long-lasting stimulation of the cyclic AMP second messenger system via activation of the Gs protein. We have previously shown that CTX in the dose range of 50-500 ng, infused bilaterally into the nucleus accumbens (N.Acc.), causes locomotor activation in rats. The present experiments extended this work. First, the behavioral effects of low doses of CTX (0, 5, 15 ng) infused bilaterally into the N.Acc., were examined. Both doses of CTX elicited a small but significant hyperactivity response (locomotion and rearing), which was apparent 2-5 days following infusion. In separate groups of rats, the motor response to intra-accumbens SKF 38393 (0.1, 1.0 μg) or quinpirole (1, 5 μg) was examined 2 days following intra-accumbens CTX (0, 5, 15 ng). The response to quinpirole was completely suppressed in CTX-treated rats. The response to SKF 38393 was affected in a more complex manner; the response to the high dose was suppressed, whereas the response to the low dose was first enhanced and then inhibited. In a second experiment, rats were trained to associate a stimulus (light-click) with food reward. Subsequently, they learned to lever-press for presentation of the stimulus (responding for conditioned reinforcement). Bilateral intra-accumbens infusion of CTX (100 ng/1 μl) induced a significant increase in lever pressing that lasted approximately 5 days, compared with both their own baseline and a control group infused with saline. As a positive control, the same groups of animals were administered the stimulants pripradrol (2.5, 5 mg/kg) and cocaine (10, 25 mg/kg) at later time points; these compounds also enhanced responding for CR. These results suggest that the cyclic AMP system with nucleus accumbens neurons modulates spontaneous and drug-induced locomotor activity, as well as reward-related processes.

662.6

PARAMETRIC ANALYSIS OF IN VIVO STIMULATED NUCLEUS ACCUMBENS DOPAMINE EFFLUX MEASURED BY FAST CYCLIC VOLTAMMETRY AT LEVELS TYPICALLY USED IN RAT SELF-STIMULATION PROCEDURES.
J.E.G. Williams*, Department of Psychology, Eastern Illinois University, Charleston, IL 61920.

Extracellular nucleus accumbens (NAcc) dopamine (DA) levels are increased by electrical stimulation of the ventral tegmental area (VTA). VTA self-stimulation is routinely used to maintain responding in behavioral procedures. Fast cyclic voltammetry (FCV) at carbon fibre electrodes measured the effect of electrically stimulated NAcc DA efflux in anesthetized rats with a time resolution of 10 ms (Millar et al 1992). FCV has advantages in terms of time and spatial resolution over other methods of measuring transmitter release. Single fibre microelectrodes (active surface 7μm dia.; length 20-30 μm) were implanted into the NAcc, and bipolar wire stimulating electrodes implanted into the VTA. Prior to implant, FCV electrodes were electrochemically calibrated *in vitro* with 1 μmol DA relative to a Ag/AgCl reference electrode, in 0.1 Molar phosphate buffered 0.9% w/v NaCl solution, pH 7.4. The FCV potential consisted of a 1.5 cycle, 100 Hz triangular ramp scanning between -1.0 and +1.4V relative to the Ag/AgCl reference with a scan rate of 480 v/s applied at 2 Hz. Signals were fed into a Nicolet 310 storage oscilloscope, and measurement made from hard copies of signals. FCV signals were recorded following VTA constant current stimulation presented at levels typically used in self-stimulation procedures. Results show that stimuli presented to the VTA at levels around those used in self-stimulation are correlated with DA efflux in the NAcc. Therefore, this FCV technique is useful for functional analysis of real-time neurochemical events that maintain motivated responding.

662.7

DOPAMINE RELEASE WITHIN THE NUCLEUS ACCUMBENS ELICITED BY SEX-RELATED OLFACTORY CUES: EFFECTS OF SEXUAL EXPERIENCE. J.B. Mitchell*. Dept. of Psychology, Boston College, Chestnut Hill, MA 02167.

Exposure to primary incentive stimuli, such as food or sex-related cues, leads to an increase in extracellular dopamine (DA) concentrations within the nucleus Accumbens. The current experiments investigated the effect of sexual experience on the neurochemical response to sex-related olfactory cues (bedding from cages that housed estrus female rats). Male rats were tested with each of two primary incentive stimuli, a highly palatable food and sex-related olfactory cues while sexually naive and again after a test for sexual behaviors. During presentation of these stimuli, extracellular DA concentrations were measured within the nucleus Accumbens using high speed chronoamperometry. The electrochemical signal was obtained by applying a +0.55 V pulse, relative to a Ag/AgCl reference electrode, to a carbon fiber electrode, at a rate of 5 Hz. While sexually naive, presentation of either food or sex-related olfactory cues reliably elicited an increase in the electrochemical signal. After sexual experience, the response to sex-related olfactory cues was significantly enhanced, whereas the response to food did not differ from that obtained on the earlier test. These results indicate that naturally rewarding stimuli increase activity within the mesolimbic DA system. Furthermore, the augmented response to sex-related olfactory cues after sexual experience suggests that changes in DA release within the nucleus Accumbens are stimulus specific and depend, at least in part, on the animal's previous experience.

662.8

INDIVIDUAL DIFFERENCES IN SUGAR CONSUMPTION PREDICT INDIVIDUAL DIFFERENCES IN AMPHETAMINE-STIMULATED DOPAMINE OVERFLOW IN THE POSTERIOR-MEDIAL ACCUMBENS. T.L. Sills* and J.N. Crawley, Section on Behavioral Neuropharmacology, NIMH, Bethesda, MD 20892

Rats exhibit significant individual differences in their consumption of sugar and in their response to amphetamine (AMP) treatments. Intrinsic variation in the functioning of the mesolimbic dopamine (DA) system is one potential mechanism underlying the expression of these individual differences. The present experiment examined the relationship between sugar consumption and the DAergic and locomotor responses to AMP (1.75 mg/kg, i.p.). Rats were divided into LOW and HIGH sugar feeders based on a median split of their sugar consumption in response to a saline injection. *In vivo* microdialysis was used to assess AMP-stimulated DA overflow in the posterior-medial nucleus accumbens (Acc) in LOW and HIGH rats, and concomitant measures of locomotor activity were obtained. There was a significant correlation between sugar consumption and AMP-stimulated DA overflow in the posterior-medial Acc. HIGH rats exhibited significantly larger increases in AMP-stimulated DA overflow than LOW rats. HIGH rats also exhibited significantly higher levels of Acc-DA overflow immediately after handling and entry into the novel test chamber, suggesting a possible difference between LOW and HIGH rats in the Acc-DAergic response to mild stress. LOW and HIGH rats did not exhibit differences in locomotor activity either in response to being handled or in response to AMP. These results indicate that the propensity to ingest sugar is a predictor of the Acc-DAergic response to AMP treatment and mild stress.

662.9

EFFECTS OF D-AMPHETAMINE AND PHENCYCLIDINE ON BEHAVIOR AND EXTRACELLULAR CONCENTRATIONS OF NEUROTENSIN AND DOPAMINE IN THE VENTRAL STRIATUM AND MEDIAL PREFRONTAL CORTEX OF THE RAT. T.H. Svensson, P. Hertel, J.M. Mathé, G.G. Nomikos, M. Iurlo, B. Ulfhake* and A.A. Mathé, Dept Physiol & Pharmacol, Div Pharmacol, Karolinska Institutet, 171 77 Stockholm, SWEDEN

The effects of phencyclidine (PCP; 2.5 mg/kg, s.c.) and d-amphetamine (AMPH; 1.5 mg/kg, s.c.) on neurotensin-like immunoreactivity (NT-LI) and dopamine (DA) in the ventral striatum (vSTR) and the medial prefrontal cortex (mPFC) were studied in freely moving rats using microdialysis. The effects of PCP and AMPH on locomotor activity were also analyzed. PCP, but not AMPH, caused a significant 156% increase of NT-LI levels in the vSTR which was relatively short lasting (< 2 h.) In contrast, both drugs significantly increased NT-LI levels in the mPFC by almost 100% during the same period. PCP and AMPH also significantly increased DA levels in the vSTR by 83% and 364%, respectively. However, the peak effect of PCP on DA appeared later than that of AMPH, i.e. at 150 min and 60 min, respectively, after drug administration. Also in the mPFC both PCP and AMPH significantly increased DA levels by 98% and 284%, respectively. Generally, effects on DA levels of both PCP and AMPH were, in contrast to their effects on NT-LI levels, more long-lasting, i.e. of 3-4 hours duration. Behaviorally, AMPH produced a more pronounced, general activation than PCP, with a faster onset of action, i.e. within 30 vs. 90 minutes after administration. However, both drugs produced long-lasting effects on the spatial organization of behavioral activity, which lasted for 3-4 hours. Thus, the more pronounced behavioral stimulation by d-amphetamine vs. PCP in the rat may largely be explained by its more potent DA releasing effect in brain. Initial behavioral suppression by PCP, e.g. of rearing, as well as its rather poor locomotor stimulant action might relate to release of NT in the vSTR. The long-lasting, behavioral disorganization by both PCP and AMPH may, however be related to increased release of DA rather than NT in the mesolimbocortical areas.

662.10

D2 ANTAGONISTS INDUCE STRIATAL FOS-LIKE IMMUNOREACTIVITY IN RATS DEPLETED OF STRIATAL DOPAMINE AS WEANLINGS, BUT NOT AS ADULTS.

M. Sandstrom*, M. Sarter, and J.P. Bruno, Dept of Psychology and Neuroscience Program, Ohio State University, Columbus OH 43210

Rats depleted of striatal dopamine (DA) with 6-OHDA as adults exhibit profound motoric deficits for several weeks after the lesion. Once recovered, these rats are supersensitive to the motoric effects of low doses of D1 or D2 antagonists. Administration of D2 antagonists to rats unilaterally depleted of DA as adults induces Fos-like immunoreactivity (FLI) in the intact, but not in the depleted striatum. In contrast, rats depleted of striatal DA as weanlings (Day 20) exhibit modest motoric deficits for only days after the lesion. As adults, these rats are not supersensitive to the motoric effects of low doses of DA antagonists. We have found that D2 antagonists induce FLI in both the intact and the depleted striata in rats treated with 6-OHDA on Day 20. We interpret this lack of behavioral supersensitivity to low doses of DA antagonists, and the retention of D2 antagonist-induced FLI following the depletion in weanlings as suggesting that extracellular levels of DA remain high in the lesioned striata of these animals. Such increased extracellular DA may also account for the fact that rats depleted of DA as weanlings exhibit only modest deficits and recover from them within several days.

662.11

THE LOCOMOTOR EFFECTS OF QUINPIROLE IN THE NUCLEUS ACCUMBENS OF DEVELOPING RATS. C. Van Hartesveldt* and K. J. Frantz, Department of Psychology, University of Florida, Gainesville, FL 32611 U. S. A.

The purpose of the present experiment was to analyze the locomotor activity of developing rats in response to various doses of the dopamine D2 subfamily agonist, quinpirole, injected directly into the nucleus accumbens. Ten-, 20-, and 30-day-old rats were implanted bilaterally with guide cannulae in the cortex above the nucleus accumbens. On the next day, rats were injected with 0.25ul of distilled water containing one of several doses of quinpirole (0.00, 0.01, 0.1, 1.0, 10.0, 20.0, 40.0 ug). Immediately after the injection, locomotor behavior was recorded for 2 hr.

The responses of 10-day-old pups were highly variable, but generally all doses of quinpirole increased activity throughout the test session. In 20-day-old pups, low doses of intra-accumbens quinpirole did not alter locomotion. However, the high doses decreased activity early in the test session and increased activity later in the session; this pattern is known as the biphasic locomotor response. The finding that high doses of quinpirole were required to decrease activity in 20-day-old rats indicates that, at this age in the nucleus accumbens, locomotor suppression is not a "low dose phenomenon" and therefore is not likely to be mediated by autoreceptors. In contrast, a low dose of quinpirole given to 30-day-old rats did decrease activity early in the test session, thereby inducing the classic low dose agonist-induced locomotor suppression. High doses induced the biphasic response in 30-day-olds. Dopamine autoreceptor function, D2 and D3 receptor activation, and drug diffusion are possible determinants of these behaviors.

662.12

PAW PREFERENCE, TURNING BEHAVIOR AND DOPAMINE FUNCTION IN THE COLLINS HI AND LO LATERALIZED MOUSE STRAINS. D.M. Nielsen,* J.N. Carlson, K.E. Visker, R.W. Keller, Jr. and S.D. Glick, Dept. of Pharmacology and Neuroscience, Albany Medical College, Albany, NY 12208

Mice exhibit a paw preference that is not task specific and is consistent upon repeated measurement. The Collins HI and LO lines are two populations of mice that have been selectively bred to differ markedly in the degree of paw preference. They represent a unique genetic model of functional cerebral lateralization. Rotation (or circling) behavior in normal unlesioned animals reflects an endogenous lateralization of the functioning of brain dopamine (DA) systems. In the present studies rotational behavior and lateralized brain DA neurochemistry were assessed in the Collins HI and LO lines of mice. Confirming Collins' data, HI strain mice exhibited a stronger paw preference than LO strain mice. HI strain mice also showed more net turns and a stronger percent directional preference during nocturnal tests of spontaneous rotation. As a population, HI mice also had a predominantly leftward turning preference. Neurochemical differences were also apparent between the strains. DA and its metabolites were measured in the medial prefrontal cortex (PFC), nucleus accumbens (NAS) and striatum. The lines differed on certain measures of DA utilization in the PFC and NAS. Side, paw preference, turning preference, sex and strain interacted in a complex way to determine measures of DA utilization in the striatum. (supported by MH 45539)

662.13

RELATION BETWEEN ELECTRICALLY EVOKED ROTATIONAL BEHAVIOR AND DOPAMINE OVERFLOW IN THE CAUDATE NUCLEUS AND NUCLEUS ACCUMBENS. L. T. L. Tran-Nguyen* and E. Castañeda. Arizona State University, Tempe, AZ 85287-1104.

Male Sprague-Dawley rats received a unilateral bipolar electrode in the medial forebrain bundle and bilateral microdialysis probes in the caudate nucleus (CN) or nucleus accumbens (NAc) and 24 hr later were tested for 2 days. On day 1, rats received monopolar electrical stimulation at 100μA or 200μA (50 Hz, 10s trains, 1-min ISI) for a 20-min period. On day 2, rats received stimulation at the other intensity. Next, electrical stimulation (200μA) was applied in the presence of tetrodotoxin (TTX; 5μM) infused through the microdialysis probe ipsilateral to the electrode. Extracellular overflow of dopamine (DA), 3,4-dihydroxyindoleacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were measured during resting state and electrical stimulation. Quarter turns contralateral and ipsilateral to the stimulated side were measured throughout microdialysis testing. Contralateral turns occurred during electrical stimulation and were intensity-dependent. TTX infusion attenuated this behavioral response. DA and DOPAC overflow were enhanced bilaterally during electrical stimulation in the CN in an intensity-insensitive manner and only at the higher intensity in the NAc. HVA and 5-HIAA levels showed a delayed increase in response to the electrical stimulation that peaked 20 to 40 min following stimulation. TTX infusion decreased monoamine overflow in the treated hemisphere. The ability to use this technique to study functional changes in DA metabolism will be discussed.

662.14

ROTATIONAL BEHAVIOR IN INTACT RATS FOLLOWING INTRA-STRIATAL INJECTIONS OF DOPAMINERGIC DRUGS. I.D. Smith* and R.J. Beninger. Dept. Psychology, Queen's Univ., Kingston, Canada.

Imbalances in striatal dopamine (DA) following unilateral 6-OHDA lesions produces rotational behavior as a result of receptor supersensitivity. Motor asymmetry due to manipulation of DA receptors in rats without DA depletions is rarely studied. Rotation caused by glutamate (GLU) receptor agonists (NMDA, kainate, AMPA and ACPD) has been studied in normosensitive rats and is dependent on DA receptor tone. It is unclear however, whether the behavioral effects of DA agonists depend on GLU receptor stimulation.

Cannulated rats received 0.5μl injections into the dorsal striatum. The direct DA agonist apomorphine (0.67, 6.7, 66.7mM) failed to caused rotation. In addition, neither the D1 agonist SKF 81297 (1, 10, 100mM), the cAMP-analogue Sp-cAMPS (0.11, 1.1, 11.2mM), nor the D2 agonist quinpirole (0.78, 7.8, 78.2mM) affected turning. In contrast, the DA releaser amphetamine (AMPH) (1.1, 10.9, 108.7mM) caused contralateral circling. This effect was reversed by co-injection of the D1 antagonist SCH 23390 (3.1mM), but was not significantly reduced by the D2 antagonist eticlopride (5.3mM). Finally, AMPH-induced rotation was reversed by TTX co-injections (100μM) but was not blocked by the NMDA receptor antagonist AP7 (0.88μM) or the kainate/AMPA antagonist CNQX (500μM).

These data suggest that post-synaptic stimulation of striatal DA receptors by exogenous drugs (apomorphine, SKF 81297, quinpirole) does not cause behavioral asymmetry, whereas releasing endogenous stores of DA (AMPH) will result in turning away from the injection. This rotation appears to depend on D1 receptors (SCH 23390 block) and may occur through an increase in striatal discharge (TTX block), but does not seem to require intact GLU neurotransmission (AP7, CNQX failure to block). (Supported by NSERC)

662.15

A COMPARISON OF THE LOCOMOTOR STIMULANT EFFECTS OF D1-LIKE AGONISTS IN MICE. P. Terry* and J.L. Katz[1]. [1]Psychobiology Section, NIDA Addiction Research Center, P.O. Box 5180, Baltimore, MD, USA, and School of Psychology, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

Agonists at D1-like receptors are often reported to produce only mild stimulation of locomotor activity in rodents. However, a recent study described a profound, long-lasting locomotor stimulant effect of the prototypical D1-like agonist SKF 38393 in C57/Bl6J mice (Tirelli and Terry, 1993, *Psychopharmacol.*, **110**: 69). The present study compared effects of five D1-like agonists on locomotor activity in non-habituated, Swiss-Webster mice. Each drug was injected IP (0.1 - 100 mg/kg and vehicle; independent groups) and locomotor activity was monitored in photobeam arenas for 3 hours post-injection. Four of the drugs (SKF 82958, SKF 81297, SKF 77434 and SKF 75670) produced strong stimulant effects over the dose range 0.1 - 10 mg/kg, and these persisted for at least two hours. At 100 mg/kg, each drug produced a biphasic effect: locomotor inhibition followed by enhanced activity. The fifth drug, SKF 38393, only produced the high-dose biphasic effect; there was negligible locomotor stimulation at the lower doses. However, SC injection of SKF 38393 produced stimulant effects across the dose range, and these continued beyond three hours. For the five agonists, there was no relationship between reported efficacy in stimulating adenylyl cyclase activity and efficacy in stimulation of behavior, nor was there any clear association between reported receptor affinity and behavioral potency. The results confirm the stimulant profile of D1-like agonists and further demonstrate a dissociation between certain pharmacological characteristics of these drugs and their behavioral effects. The results also indicate that effects of SKF 38393 are not always characteristic of other D1-like agonists.

662.16

A NOVEL SYSTEM FOR SIMULTANEOUS MEASUREMENT OF LOCOMOTOR ACTIVITY DURING MICRODIALYSIS EXPERIMENTS IN FREELY-MOVING RATS. D.L. Alexoff, S.L. Dewey, C. -E. Chen, D. L. Tedeschi, R. Straughter-Moore, D. D'Emilia, J.S. Fowler* and S.J. Gatley. Chem and Med Dep'ts, Brookhaven National Laboratory, Upton, NY 11973

Microdialysis can accelerate the biological characterization of new drugs or neuroactive chemicals by measuring changes in extracelluar neurotransmitter concentrations following pharmacologic challenge. Behavioral data on freely moving animals during microdialysis, however, is often not collected due to the complication and expense of interfacing standard commercial behavioral systems to new microdialysis equipment. We describe an inexpensive system for monitoring rat locomotor activity simultaneously during microdialysis experiments. The system is based on the gimbaled tethering arm in Bioanalytical Systems CMA/120 Awake Animal System. An optical proximity detector (SmartEye, TRITRONICS, Tampa, FL) was mounted opposite the end of the gimbaled arm so that excursions of the arm caused by the animal's movements would alternatively reflect or let pass the detector's infra-red beam. The digital output of the detector was interfaced to an IBM PC programmed to count both positive and negative arm deflections during the experiment. Locomotor counts were summed over 5 min. intervals and stored. Data was collected using 4 time windows (0, 1, 3 and 5 sec.) to evaluate the detector's selectivity to rat head movements, such as grooming or sniffing, compared to gross movements. Locomotor data collected after amphetamine was consistent with the well-characterized amphetamine-induced locomotor response. This system is suitable for assaying locomotor responses to drug challenges during microdialysis experiments. We have applied this system to potential new radiopharmaceuticals including novel monoamine uptake inhibitors (Pan, et al., Eur. J. Pharm., 1994) and new serotonergic ligands. Supported by DOE/OHER, NINDS, NIMH 49165, NARSAD and BNL-LDRD.

662.17

QUINPIROLE MODULATION OF ACTIVITY LEVELS AND EXPLORATORY BEHAVIOR IN RATS WITH FORNIX TRANSECTIONS. B. Osborne*, A.C. Dukowicz, D.A. Hutcheson, W. I. Maris and L.G. Adams. Dept. of Psychology, Middlebury College, Middlebury, VT 05753.

The ability of the dopamine agonist, quinpirole, to modulate the exploratory behavior and activity levels of control rats and rats with fornix transections was examined under different dose levels and basal activity levels. Control rats and rats with fornix transections were given either saline, 0.03 mg/kg quinpirole, or 0.5 mg/kg quinpirole, i.p., 15 mins. prior to exploration of an enriched novel environment. Following this first experience, rats were habituated to the environment and tested again under the same drug conditions. Under both conditions, latency to move, activity levels and the frequency and duration of rearing and object interactions were recorded. With saline injections, fornix transected rats were more active, had more frequent object interactions, less rearing, and the durations of behaviors were shorter. At the .03 mg/kg dose level, quinpirole reduced activity of all groups and returned the activity levels of fornix transected rats back to normal levels. At 0.5 mg/kg, quinpirole reduced the levels of fornix transected rats but to a lesser extent. Controls were not affected on initial exposure, but both control and fornix transected rats failed to habituate under the higher dose level. Exploratory behavior was also affected but to a lesser extent. The results are discussed in terms of pre-and post-synaptic effects at different dose levels, and dopamine and glutamate actions at nucleus accumbens.

662.18

STIMULATION OF DOPAMINE RECEPTORS IN THE MEDIAL PREFRONTAL CORTEX MEDIATES INHIBITION OF SPONTANEOUS LOCOMOTOR ACTIVITY. R. A. Radcliffe and V. G. Erwin*. School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO 80262

It is well established that stimulation of dopamine receptors (DAR) in the nucleus accumbens (Acb), a major limbic DA terminal field, initiates increases in locomotor activity. The medial prefrontal cortex (mPFC), also an important DA terminal field, is thought to regulate DA functions in the Acb. The locomotor response to DA stimulation in the mPFC, however, has not been well characterized. We have previously demonstrated that application of the selective DA uptake blocker GBR-12909 into the mPFC of LSP/IBG mice causes a dose-dependent decrease in locomotion followed by a return to control values. The intent of the present study was to determine which DAR subtype(s) is responsible for the observed biphasic response and if application of GBR into the mPFC mediates altered DA activity in the mPFC or in the Acb. Bilateral cannula guides were implanted into the mPFC of LSP/IBG mice. 24 hours post surgery, the subjects were injected bilaterally with either GBR, the DAR-D_1 antagonist R(+)-SCH-23390, the DAR-D_2 antagonist epidepride, or a combination of drugs. Distance traveled was monitored in an automated open field apparatus. Doses of SCH (1-100 pmol) alone had no effect on locomotor activity but simultaneous injection (10 pmol) with GBR (0.1-1000 pmol) attenuated GBR-induced inhibition. Epidepride (0.1-100 fmol) alone produced a dose-dependent decrease in locomotor activity, but simultaneous injection with GBR had no effect on the response to GBR. Alteration of DA metabolism was estimated from tissue levels of DA and DA metabolites, determined with HPLC coupled to EC detection, in the mPFC and Acb after unilateral injections of doses of GBR. Preliminary evidence indicates that 5 min post injection, GBR caused the ipsilateral mPFC HVA/DA ratio to increase above contralateral values at low doses and return to control at high doses. Ipsi- and contralateral DOPAC and DA levels were not different in the mPFC. In contrast, at this same time point, GBR had no effect on DA, HVA, or DOPAC levels on either side of the Acb. The results of these studies suggest that an increase in synaptic DA in the mPFC is responsible for depressed locomotion and that this response is mediated by DAR-D_1. Furthermore, while blockade of DAR-D_2 is able to suppress locomotion, this effect is not additive to the GBR response. (This work was supported, in part, by USPHS grants DA01717 and AA07330.)

662.19

CHANGES OF THE ACOUSTIC STARTLE RESPONSE IN RATS AFTER LOCAL INJECTION OF PERTUSSIS TOXIN INTO THE VENTRAL TEGMENTAL AREA. J. Zhang, J. A. Engel, S. Hjorth and L. Svensson*. Dept. Pharmacol., Göteborg Univ., Göteborg, Sweden.

The effect of local injection of pertussis toxin (PTX) into the ventral tegmental area (VTA) on the acoustic startle response (ASR) in rats was investigated. The PTX treatment caused only minor effects of its own on ASR magnitude or prepulse inhibition (PPI) of ASR magnitude. However, systemic treatment with the indirect DA receptor agonist, amphetamine (2 mg/kg, s.c.), caused a significant increase of ASR magnitude and a significant disruption of PPI in PTX-treated rats while no such effects were observed in sham-treated rats. Treatment with the direct DA receptor agonist, apomorphine (2 mg/kg, s.c.), caused a significant disruption of PPI, an effect that was observed in both PTX- and sham-treated rats. Treatment with the 5-HT_{1A} receptor agonist, 8-OH-DPAT (0.5 mg/kg, s.c.), did not affect PPI in either group but caused a marked increase of ASR magnitude in sham-treated rats. Interestingly, this effect was blocked in PTX-treated rats. The present results suggest that local injection of PTX into the VTA causes an increased sensitivity to the behavioural effects of psychostimulants on acoustic startle and may also suggest that intact midbrain 5-HT_{1A} receptors are essential for the effect of 5-HT_{1A} agonists on acoustic startle.

662.20

EFFECTS OF REWARD AND STRESS ON IN VIVO HYDROXYLATION OF TYROSINE AND TRYPTOPHAN IN RAT NUCLEUS ACCUMBENS: A MICRODIALYSIS STUDY. D. Nakahara* and M. Nakamura. Department of Psychology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan

Microdialysis studies have recently shown that serotonin metabolism in the nucleus accumbens is equally enhanced by rewarding and stressful stimuli, while the release and metabolism of dopamine in this region is differentially affected by these two stimuli, with a larger enhancement in rewarding than stressful situations. In the present study, we applied the microdialysis technique to assess the changes in the synthesis of dopamine and serotonin in response to rewarding and stressful manipulations by measuring accumulation of 3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) after the local infusion of an aromatic L-amino acid decarboxylase inhibitor, NSD-1015 (3-hydroxybenzylhydrazine dihydrochloride), through the dialysis membrane. The rate of accumulation of DOPA and 5-HTP was utilized as an index of the in vivo hydroxylation of tyrosine and tryptophan, respectively. Self-stimulation of the medial forebrain bundle was used as a rewarding manipulation and immobilization as a stressful manipulation consisted of taping their limbs to a wood board with adhesive tape.

Self-stimulation caused a significant increase in both DOPA and 5-HTP accumulations. On the other hand, immobilization stress increased 5-HTP accumulation without affecting DOPA accumulation.

Taken together with previous data, our results suggest that dopamine activity in the nucleus accumbens may be more responsive to reward rather than stress, and that serotonin activity in this region may be responsive to stress as well as reward.

663.1

THE SEROTONIN SYNDROME IN THE MOUSE. R.J.Blanchard*, G. Griebel, M.M.R. Brush, Jennet Lee, and D.C. Blanchard, Pacific Biomedical Research Center, Univ. of Hawaii, Honolulu, HI 96822

The effects of selective activation of central 5-HT_{1A} receptors on the behavior of mice have not been clearly defined. Yamada et al. (1988) found that the 5-HT_{1A} receptor full agonist 8-OH-DPAT induced a distinct 5-HT syndrome, whereas Goodwin and Green (1985) reported that in mice the drug did not induce any distinct behavioral alteration.

Behavioral effects of 8-OH-DPAT (0.5-10m g/kg, i.p.) were examined in mice in a home cage setting. Vehicle controls displayed an initial activity that declined gradually over the observation period, providing a shifting baseline for evaluation of 8-OH-DPAT effects. During initial segments of the test period, 1 and 10 mg/kg blocked rearing and hanging on the wire mesh. Eating was increased at 10 mg/kg. Grooming was inhibited and Straub tail response increased at all doses. In the earliest post injection period (10 min) 8-OH-DPAT, at low and moderate doses only, produced rapid, ballistic-type forelimb movements ending near the sides of the mouse's head. Also, in an oval runway permitting limitless forward progression, moderate and high doses produced a distinct suppression of forward locomotion which exhibited itself as periods of immobility, tight rotating and circling.

These data provide some puzzling differences from the effects of 8-OH-DPAT in rats, which include continuous forward locomotion and forepaw treading when forward locomotion was interrupted. The present finding of opposite patterns in the mouse suggests the value of investigation of species differences in response to compounds which act at the 5-HT_{1A} receptor.

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663.2

THE EFFECT OF EXERCISE AND L-TRYPTOPHAN ADMINISTRATION ON EXTRACELLULAR SEROTONIN METABOLISM IN RAT HIPPOCAMPUS. R. Meeusen*, K. Thorré, S. Sarre, K. De Meirleir, G. Ebinger and Y. Michotte, Dept. Human Physiology and Sportsmedicine, Vrije Universiteit Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium

The purpose of this study was to examine the effects of L-TRP administration in combination with exercise on extracellular concentrations of 5-HT and 5-HIAA in rat hippocampus using microdialysis. The day before the experiment, after the microdialysis probe was implanted, male albino wistar rats were placed on the treadmill and stayed there until the end of the experiment. The following day, after stable baseline values were obtained. L-TRP (50 mg/kg i.p.) or saline was administered in a single dose. One hour later the animals ran for 60 min at a moderate speed (12 m/min) on a treadmill. Administration of L-TRP slightly increased extracellular concentrations of 5-HT and 5-HIAA. During and following exercise the 5-HT and 5-HIAA concentrations significantly increased (+ 120 %) and stayed above baseline during the following two hours. Extracellular 5-HT and 5-HIAA concentrations were significantly different between the saline-exercise and the L-TRP-exercise animals. Our results suggest that after precursor loading 5-HT metabolism and release is increased due to exercise.

663.3

CHANGES IN EXTRACELLULAR BRAIN SEROTONIN DURING THE LIGHT/DARK TRANSITION:RELEASE IS CORRELATED WITH BEHAVIORAL ACTIVITY RATHER THAN THE CIRCADIAN CYCLE B.L. Jacobs* and L.E. Rueter Prog. Neurosci.,Dept. Psychology, Princeton Univ., Princeton, NJ 08544

The degree of site specificity with which serotonin is released in the forebrain and the physiological conditions under which release is altered are still unclear. The present study was designed to address these issues in the context of changes across the daily transition from light to dark, by employing in vivo microdialysis in the freely moving rat. Cannulae were implanted in the anterior dorsolateral hippocampus, the corpus striatum, the amygdaloid complex, and the frontal cortex in male Sprague-Dawley derived rats (two different sites per animal, all six site combinations used). After one week of recovery, concentric microdialysis probes were inserted in both sites and perfused simultaneously. Sampling began three hours prior to lights off and at least twelve hours after probe insertion. Samples were taken every half hour for three hours of light and then two hours of dark. Each session was videotaped and scored for time spent in alert waking. Results indicate that serotonin release increases significantly during the first half hour of dark phase in all areas tested (hippocampus 43.7%, striatum 11.3%, amygdala 16.8%, frontal cortex 19.5%). The increase in hippocampus is significantly greater than the other sites. Release was positively correlated with alert waking time (hippocampus r=.87, striatum r=.61, amygdala r=.88, frontal cortex r=.91). When matched for activity levels within subjects, serotonin release in the light essentially equaled release in the dark (activity> 15 min: light 121% of baseline,dark 119%; activity< 5 min: light 97% of baseline, dark 89%). These results show that, in agreement with electrophysiological studies, serotonin release is correlated with behavioral activation rather than the light-dark cycle per se, and that there can be specificity of degree of release in target areas of the serotonergic system (i.e. greater release in the hippocampus). Supported by grants from AFOSR and NIMH.

663.4

SEROTONIN RELEASE DURING STRESSFUL AND NON-STRESSFUL CONDITIONS: IS THE SEROTONERGIC SYSTEM SPECIFICALLY STRESS-ACTIVATABLE? L.E. Rueter* and B.L. Jacobs Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

Neurochemical studies indicate that stressors increase release and/or turnover of serotonin while electrophysiological studies indicate that stressors do not increase serotonergic neuronal activity above levels seen during spontaneous active waking. The present study was designed as an attempt to reconcile these apparently disparate literatures using in vivo microdialysis to measure serotonin in various forebrain sites. We also employed two important experimental design controls: stress exposure during the rat's dark or active phase and direct comparison of the stress response to a non-stressful condition containing somewhat similar behavioral/activational components. (See previous abstract for experimental details.) Sampling began a half hour into dark phase; sample time was 30 minutes. The baseline or control condition was taken as the 90 minutes prior to manipulation. Each rat was exposed to two conditions of the following manipulation sets: 1. tail pinch(tp), tp with food, and food; and 2. swimming and floating. Order of presentation was varied. Results indicate that

CONDITIONS (% increase from baseline; all n's > or = 10)

SITE	tp	tp w/food	food	swim	float
hippocampus	50.6%	41.0%	37.5%	20.3%	26.8%
striatum	28.0%	32.2%	26.1%	15.2%	12.8%
amygdala	36.3%	55.7%	43.3%	36.0%	40.4%
frontal cortex	33.2%	44.8%	27.0%	57.1%	35.7%

serotonin release during tp, tp with food, and food was increased in each area to approximately the same degree. Serotonin release was also increased during swimming and floating in each area to approximately the same degree. These results suggest that under these conditions serotonin is released in the forebrain in a fairly nonspecific manner and that the serotonergic system is not specifically stress activatable but rather that serotonin release is best correlated with behavioral activation/motor activity. Supported by grants from AFOSR and NIMH.

663.5

CLONING THE ECDYSONE-RECEPTOR FROM THE AMERICAN LOBSTER, *Homarus americanus*. A. Quinones-Hinojosa* & E. A. Kravitz, Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

The steroid ecdysone is the molting hormone in crustaceans and insects. Ecdysone has been shown in insects to serve important roles in the transformation of the larval CNS to that of the adult. Ecdysone acts by binding to a cellular receptor protein, which is translocated to the nucleus where it binds to multiple target genes. Ecdysone levels fluctuate over the molt cycle in lobsters with a large peek seen in the D2-D3 period (Snyder and Chang, 1991). Aggressive behavior also varies over the molt cycle in lobsters (Tamm and Cobb, 1978). Early in the cycle (post molt period) animals show little aggression, while shortly before the next molt (premolt) animals become highly aggressive. Our studies suggest that serotonin is involved in aggressive behavior in lobsters. Certain serotonergic neurons show elevated levels of the amine in the premolt period. In beginning to explore the possibility that ecdysone, serotonin and aggression are linked in lobsters, we are attempting to clone the lobster ecdysone receptor.

First stage larval lobster were used for these studies. mRNA was isolated from whole animals and cDNA was synthesized. Oligonucleotides were designed from highly conserved regions of the cDNA sequences of Drosophila ecdysone-receptor genes. Using a PCR strategy, amplified pieces of DNA were cloned and later sequenced by the Sanger method. A 535 bp fragment was isolated that showed 70% sequence homology to the ecdysone-inducible genes E75A and B from D. melanogaster. We are attempting to isolate and characterize the full length cDNAs for these transcription factors and will continue a search for the ecdysone receptors themselves. As the various molecular entities are isolated we will design probes to ask whether ecdysone-receptors or related genes are expressed in nerve cells containing the neurotransmitter serotonin.

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663.6

IN-VIVO VOLTAMMETRY IN THE NERVOUS SYSTEM OF INVERTEBRATES M. Hörner, F.W. Schürmann* and E.A. Kravitz, Harvard Medical School, Dept. of Neurobiology, 220 Longwood Ave., Boston 02115, MA,

There is growing evidence that biogenic amines like serotonin (5HT) after release from neuronal terminals can have both synaptic and non-synaptic influences (Fuxe & Agnati, 1991; Volume Transmission In The Brain; Novel Mechanisms For Neural Transmission). In the latter case diffusion through extracellular space allows neuroactive substances to affect a wide target area and not only immediate adjacent post-synaptic sites. 5HT has been shown to exert strong influences on behavior in insects and lobsters. In these species possible paracrine release sites have been identified by immunocytochemistry.

In order to measure dynamic changes of extracellular 5HT levels with high temporal and spatial resolution we have been using chronoampèrometry (0.5 V, 5-10 Hz, IVEC, Medical Systems Corp.) with fine diameter carbon-fibre glass electrodes coated with Nafion™. Recordings are made in regions shown by immunocytochemistry to be high in 5HT. The identification of electroactive species is based on the substance-specific ratios of redox-currents.

In the cricket brain, site-specific release of serotonin-like signals is observed in the region of the central body during aversive behavior. The time course of the voltammetric signals, when related to the ongoing behavior, suggests diffusion of 5HT from terminals within the central body, a neuropil area containing high amounts of 5HT. In lobsters we have compared signals generated in physiologically identified serotonergic, octopaminergic, GABAergic and glutamatergic neurons. In preliminary experiments we have measured the clearance of injected 5HT from the circulation using carbon electrodes chronically implanted into the heart. Our future goals are to measure the release of serotonin into known neurohemal areas during aggressive behavior.

Supported by the Alexander von Humboldt Foundation and NINDS

663.7

PROZAC, SEROTONIN UPTAKE AND AGGRESSION IN LOBSTERS. M. M. Orzeszyna, R. Huber[1], and E.A. Kravitz*. Dept. of Neurobiology, Harvard Med. School, Boston 02115, [1]Dept. of Neurobiology, Institute of Zoology, University of Graz, Universitatsplatz 2, Graz 8010, Austria.

Our previous studies suggest that serotonin serves important roles in aggression in lobsters. Fluoxetine (Prozac), a valuable therapeutic agent in the treatment of depression, is an effective blocker of serotonin uptake in vertebrates. With the aim of possibly using this substance in studies of aggression in lobsters, we first examined the effects of Prozac on serotonin (5-HT) uptake in lobster nervous tissue. Incubations were performed using pairs of second thoracic roots, which contain dense plexuses of serotonergic and octopaminergic neurosecretory terminals. Nerves from one side acted as controls for the other side, and 5-HT and octopamine levels were measured using HPLC with electrochemical detection. Incubation in 10^{-5}-10^{-6} M 5-HT increased the levels of 5-HT in tissues by 60% with no effect on octopamine levels. Prozac effectively blocked this increased uptake of 5-HT.

Further experiments, using ^{3}H-5-HT, supported an earlier finding (Livingstone et al., 1980) that there are likely to be two forms of 5-HT uptake: a high-affinity, saturable uptake into serotonergic neurons, and a second low-affinity, non-saturable uptake, possibly into surrounding tissues. Prozac appears to act as a competitive inhibitor of the former form of uptake alone.

When injected into living animals, Prozac (10^{-4} M) was not toxic. We also saw no immediate behavioral effect of acute Prozac treatment. 5-HT injections cause subordinate animals to be significantly more likely to engage in fights with dominants (Huber *et al.*, unpubl.). We are now testing the effects of Prozac on the 5-HT-mediated enhancement of aggressive behavior of subordinate animals and examining the effect of chronic Prozac treatment.

Supported by NINDS and the Ford Foundation.

663.8

CLONING OF AMINE HYDROXYLASE GENES FROM THE LOBSTER. X.-S. Chen, L.N. Geller, A. Kopp, R. Hawley, C. DeFranco*, and H. Potter. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

The amine hydroxylases are of particular interest because of their roles as the rate-limiting enzymes on the pathway for serotonin and catecholamine synthesis which have been shown to induce posture and behavior changes in lobster. We have isolated five independent amine hydroxylase cDNA clones including two 5'-end and three 3'-end RACE clones. Sequence analysis demonstrated that (1) The two 5'-end RACE clones are totally different from each other at their 5' untranslated regions, partially different at the beginning of the translated regions, and identical in the remainder of the translated region. (2) All of the 3'-end clones terminate at known polyA sites and are identical in their translated regions. They have identical 3' untranslated regions for a short stretch, followed by different sequences. (3) The three 3'-end clones overlapped the 5'-end clones, thus identifying a contiguous coding sequence. (4) Alignment of deduced lobster TRH with *Drosophila* TRH/PAH, rat TRH, rat PAH, and rat TH proteins showed >76% identity. Northern blot analysis of lobster polyA+ mRNA revealed three transcripts of 1.6 to 2.2 kb. The exact identity of the protein encoded by the single large open reading frame is not apparent from the sequence data, although it is clearly of an amine hydroxylase. *In situ* hybridization with the different untranslated sequences reveals different patterns of hybridization, raising the possibility that more than one enzyme is encoded by a single gene.

663.9

EXPRESSION OF THE SHAB GENE IN THE CNS OF THE AMERICAN LOBSTER. H. Schneider*, D.J. Baro, R.M. Harris - Warrick, & E.A. Kravitz, Neurobiology Dept. Harvard Med. Sch., Boston, MA 02115, Dept. Neurobiol. & Behav., Cornell Univ., Ithaca, NY 14850.

Serotonin (5HT) and octopamine (OA) appear to participate in the regulation of agonistic behavior in the American lobster, *Homarus americanus* (Kravitz, '88). Serotonin injections into the hemolymph trigger postures simlar to those seen in dominant lobsters while OA injections elicit postures similar to those seen in subordinate animals. In addition, 5HT injections into subordinate lobsters shortly after a hierarchy has been established, can alter the behavioral status of these animals and dramatically increase their fighting activity (Huber et al., unpubl.). In isolated nerve cords, 5HT and OA have opposing effects on flexion and extension motor circuits (Harris-Warrick & Kravitz, '84).

We have begun searching for long term physiological and molecular changes in the lobster ventral nerve cord that maybe correlated with dominant or subordinate status. Here we present the pattern of expression of the Homarus shab K^+-channel gene in 5HT- and OA neurosecretory cells and in postural motoneurons (MNs). Applying PCR methods we have partially cloned a Homarus shab gene that is 92% identical at the nucleotide level to the shab gene of the spiny lobster, Panulirus interruptus, and 82% identical to with the Drosophila shab gene. The mRNA for this gene is found in inhibitory and excitatory flexor abdominal MNs but not in 5HT- neurosecretory cells as demonstrated by single cell PCR. The expression pattern correlates with the physiological activities of these cells. Serotonin neurons are spontaneously active while the studied MNs are not. We plan to compare the shab expression pattern with those of the shal, shaw, and shaker forms of K^+-channels and ask how the patterns correlate with the intrinsic properties of 5HT- and OA cells in lobsters of different behavioral status. Supported by NINDS.

663.10

SEROTONERGIC MODULATION FOLLOWS CHANGES IN SOCIAL STATUS. S.-R. Yeh* and D.H. Edwards Department of Biology, Georgia State University, Atlanta, GA, 30302-4010.

Social relationships among animals influence their behavior by affecting the neural circuits and responses that control behavior. We have found that the effect of bath-applied serotonin on the responsiveness of the crayfish lateral giant (LG) tailflip command neuron changes with changes in the animal's social status. We recorded LG responses in socially isolate, dominant and subordinate crayfish before, during and after bath-application of 5-HT and 5-HT agonists. 5-HT reversibly enhanced the LG EPSPs and lowered the stimulus threshold in dominant crayfish, and reversibly inhibited the cell and raised threshold in subordinates. Tests with different vertebrate 5-HT agonists suggest that the LG neurons have at least three populations of 5-HT receptors that differ in their relative number or efficacy in dominant, subordinate, and isolate crayfish.

Dominant/subordinate social status is determined within half an hour of pairing isolated animals; 5-HT's effect on LG changed much more slowly following pairing. 5-HT-induced enhancement of LG's response in isolates decreased linearly in the new subordinates, crossed zero at 7 days pairing and became strongly inhibitory by 12 days. 8 days re-isolation restored the enhanced responses.

Supported by grants from the NIH and the NSF.

663.11

OPPOSITE EFFECTS OF SEROTONIN ON THE DENDRITES AND AXON OF A COMMAND NEURON M.J. Weissburg, B.E. Musolf, S.-R. Yeh and D.H. Edwards* Department of Biology, Georgia State University, Atlanta, GA, 30302-4010.

A neuromodulator usually has a suite of effects on a neuron that act together to increase or decrease excitability. The identification of many kinds of serotonin (5-HT) receptors, some of which have opposing effects on excitability, raises the possibility that 5-HT could have opposing actions at different locations in the same neuron. We report here such a case, where bath-applied $5x10^{-5}$M 5-HT increases the excitability of the lateral giant (LG) neuron of crayfish but also causes conduction failure in the cell's axon. 5-HT causes EPSPs in LG's initial axon segment to increase and the stimulus threshold of the spike to decrease, whereas the mid-axonal spike becomes smaller, briefer, and then fails. All these effects are reversible with saline wash. These opposing effects are seen in socially dominant crayfish, but not in social isolates, where 5-HT irreversibly enhances LG EPSPs and reduces threshold while axonal conduction is maintained. We suppose that the different effects of 5-HT on LG result from different distributions of two or more 5-HT receptors in the cell, and that this receptor pattern is specific to socially dominant crayfish.

Supported by research grants from NIH and NSF.

663.12

DO SEROTONIN 1A OR 1B RECEPTORS INFLUENCE OFFENSIVE AGGRESSION IN FEMALE HAMSTERS? M. A. Ioppa*, R. L. Meisel and K. Bunting. Department of Psychological Sciences, Purdue University, West Lafayette, IN 47907.

Selective 5-HT_{1A} receptor agonists and mixed agonists of the 5-HT_{1A} and 5-HT_{1B} receptors have been reported to decrease both intermale and maternal aggression in rats. In this experiment, we tested the effects of the specific 5-HT_{1B} receptor agonist, CGS 12066A, and the specific 5-HT_{1A} receptor agonist, 8-OH-DPAT, on interfemale aggression. For 3 weeks, ovariectomized hamsters received icv vehicle injections and an aggression pretest followed 2 days later by one of 3 icv doses (5, 10, and 20 µg) of either 8-OH-DPAT or CGS 12066A and an aggression test. Latency to attack, number of attacks, and number of uprights did not differ following any of the 3 doses of CGS 12066A or 8-OH-DPAT, nor were any side effects consistently observed. However, when 8-OH-DPAT was given ip, side effects significantly increased and latency to attack, number of uprights, and number of attacks significantly decreased. The neurochemical mechanisms underlying interfemale aggression in hamsters may be different from those regulating intermale aggression in rats and mice.

This research was supported by a grant from the National Science Foundation (IBN-9412543).

663.13

SEROTONIN CONTROLS FIGHTING BEHAVIOR IN CRAYFISH. Robert Huber, Edward A. Kravitz and Simone Helluy* Dept. Zoology, Univ. Graz; Dept Neurobiology, Harvard Medical School; Dept. Biology, Wellesley College.

When the amines, serotonin and octopamine are injected into the hemolymph of lobsters and crayfish, they trigger postures resembling those seen in dominant (serotonin) and subordinate (octopamine) animals. These initial observations led to detailed studies in crustaceans on the role of amine neurons in fighting behavior. The recent development of analytical methods for quantification of fighting behavior (Huber and Kravitz, 1995) now allows us to carry out pharmacological interventions and ask whether any important changes in behavior result.

Using crayfish (*Astacus astacus*), we first tested the effects of serotonin. Ten pairs of animals differing 30% in size were selected. Indwelling fused silica cannulae were glued into the pericardial sinus of the smaller animals. In all cases, when placed together the larger animal quickly assumed the dominant position (pre-inj). Using a syringe pump, saline was infused into the subordinate animal (control inj) resulting in little or no qualitative or quantitative changes in behavior. When the infusion solution was switched to saline containing serotonin at 3=B5g/min. (5-HT inj), fighting activity increased greatly. This slowly decayed back to the pre-injection levels after the serotonin infusion was turned off (post-inj). A multivariate statistical analysis identified the parameters of fighting that were changed by the amine injection. The likelihood that the smaller, serotonin-treated animal would withdraw, was significantly reduced, resulting in fights of increased duration. No other characteristics of fighting behavior were altered. Behavioral studies with serotonin uptake and receptor blockers are planned along with mapping studies of the nervous system for amine neuron distribution to ask whether amine neuron systems are similar in lobsters and crayfish. Supported by Austrian Science Foundation P10165-BIO and NINDS.

663.14

SOCIAL REPRODUCTIVE ROLES AFFECT CENTRAL MONOAMINES IN FEMALE *Anolis carolinensis*. Tangi R. Summers,* Amy L. Hunter, and Cliff H. Summers. Dept. of Biology, University of South Dakota, Vermillion, SD 57069.

Groups of 5 female *A. carolinensis* were housed with 1 male, but only 1 or 2 females exhibited recrudescing ovaries. These reproductively dominant females were above threshold weight (≥2.7g) at the end of 1 month; subordinate females were not. Final controls, 1 female housed with 1 male for one month, all exhibited ovarian growth; initial controls had quiescent ovaries. Brain monoamines and metabolites were quantified using HPLC coupled with electrochemical detection. Reproductively dominant females had significantly greater telencephalic 5-HIAA, and serotonergic turnover, as indicated by the ratio of 5-HIAA to 5-HT. Telencephalic DOPAC as well as the DOPAC/DA ratio are also significantly greater in dominant females compared to all other groups. The activation of serotonergic systems in reproductively dominant females is similar to subordinate males. However, DA system activation in dominant females is similar to dominant males. Activation of serotonergic systems in females may be related to submissive behaviors toward the male during competition for access to that male, whereas activation of the dopaminergic system may be associated with aggressive interactions with other females.

663.15

SEROTONIN DEPLETION INCREASES IMPULSIVE BEHAVIOR IN RATS. J.B. Richards*, L.S. Seiden. University of Chicago, Department of Pharm/Phys Sci., Chicago, Il. 60637.

Rats were required to choose between small immediate rewards and large delayed rewards. Preference for an immediate small reward over a large delayed reward is an operational definition of impulsivity (Logue, Behav Brain Sci, 11, 665-709, 1988). Rats received either whole brain 5HT lesions (LES) induced by intraventricular injection of the neurotoxin 5,7-DHT (100 ug/side, pre treatment with 30 mg/kg desipramine) (N = 8) or intraventricular injection of vehicle (CNT)(N = 11). Previously we have found that this treatment causes depletion of 5HT to less than 15% of control levels in all of the areas assayed (frontal cortex, nucleus accumbens/olfactory tubercle, striatum, septum, somatosensory cortex, amygdala, hypothalamus, and hippocampus). Dopamine and norepinephrine levels were not affected. Thirsty rats were given a choice between 200 ul of water presented after a delay or a smaller amount of water presented immediately. The amount of water available immediately was adjusted until each rat chose the small immediate amount of water and the large delayed amount of water with equal frequency (i.e. indifference point). We found the indifference points for delays of 0, 2, 4, 8, and 16 seconds in LES and CNT rats. At 0 s (no delay to the large amount) the there was no significant difference between the LES and CNT groups. At delays of 2, 4, 8, 16 s the LES rats chose smaller immediate amounts then the CNT rats. These results are consistent with the hypothesis that low serotonin is associated with increased impulsivity. (Supported by: MH-11191; RSA-10562, L. Seiden)

663.16

IMPULSIVE BEHAVIOUR IN RATS: THE EFFECTS OF DRUGS ON RESPONSE CHOICE WITH VARYING DELAYS OF REWARD. Christine Natasha Ryan and John Leslie Evenden* Dept of Behavioural and Biochemical Pharmacology, Astra Arcus, S-113 53, Södertälje, Sweden.

Impulsive behaviour is seen as a clinical symptom in several psychiatric disorders, particularly the personality disorders. To act impulsively may be (a) to act despite knowing the consequences may not be beneficial, (b) to choose between two actions without proper consideration of the consequences, or (c) to choose an action which has an immediate moderate effect, rather than one which has a delayed but potentially more beneficial effect. In man, impulsivity has been associated with low levels of serotonin (5-HT). Compatible with this finding, in rats, increased waiting capacity has been associated with increased levels of 5-HT. In the present study, hungry rats were trained in an operant chamber to choose between 1 pellet or 5 pellets. As the test session progressed, the reward of 5 pellets was progressively delayed up to a maximum of 60 seconds. Under baseline conditions the rats preferred five pellets if the delay was short, but chose 1 pellet if the delay was long. If, once the rats were trained, the delay was not increased, they preferred 5 pellets throughout the session, indicating that they were sensitive to delay of reward. Treatment with 10 & 30 mg/kg citalopram or 3 & 10 mg/kg imipramine did not affect reward choice, while treatment with 0.3 or 1.0 mg/kg amphetamine increased preference for the small reward. In contrast, the 5-HT antagonist, metergoline, enhanced selection of the large reward. These preliminary results indicate that 5-HT may have a more dynamic role than first supposed in different types of impulse behaviour. Prolonged training resulted in the adoption of a rigid strategy, which was insensitive to delay. By varying the variations in delay, flexible behaviour could be maintained for longer periods. Treatment with the 5-HT_{1A} antagonist, WAY 100635 (0.01, 0.03, 0.1 mg/kg) or with low doses of haloperidol (0.01 or 0.03 mg/kg) or carbemazepine (40 or 100 mg/kg) both of which are used for the pharmacotherapy of personality disorders, had no significant effects under these circumstances.

663.17

CHARACTERIZATION OF SEROTONIN 1B RECEPTOR CONTRIBUTION TO STARTLE AMPLITUDE AND PREPULSE INHIBITION IN WILD TYPE AND SEROTONIN 1B MINUS MICE. S.C. Dulawa [1], R. Hen [3], K. Sccarce[3], and M.A. Geyer[1,2] *. Departments of Neuroscience[1] and Psychiatry[2], University of California San Diego, La Jolla, CA 92093. Center for Neurobiology and Behavior[3], Columbia University, New York, NY 10032.

The present experiments explored the possible involvement of the serotonin 1B (5HT-1B) receptor in modulating two behaviors in mice: startle amplitude, and pre-pulse inhibition (PPI). PPI is an operational measure of sensorimotor gating, and is defined as the percent decrease in startle amplitude when a weak prepulse precedes a startling pulse. In rats, the direct 5HT-1A/1B agonist RU24969 reduces both startle amplitude and PPI. As yet, no highly specific 5HT-1A or 5HT-1B antagonists are available to explore the individual contribution of each receptor to these behaviors. We compared PPI in wild-type 129sV and homozygous 5HT-1B-minus 129sV mice in a startle response paradigm in which some 120 dB acoustic pulses were preceded by 2, 4, or 8 dB prepulses, above a 65 dB background. A potentiation of PPI was found in 5HT-1B-minus mice relative to wild types, suggesting that the 5HT-1B receptor modulates PPI ($p<.001$). In addition, startle amplitude was greater in wild-type than in 5HT-1B-minus mice. In subsequent studies, mice received 0 or 10 mg/kg BW intraperitoneal injections of RU24969. Analysis revealed a main effect on startle amplitude, with higher startle amplitudes observed in wild-type than in 5HT-1B-minus mice. There was also a group by drug interaction, as RU24969 reduced startle amplitude in wild-type mice, but did not alter startle amplitude in 5HT-1B-minus mice. 5HT-1B-minus mice demonstrated more PPI than wild-type mice at the 0mg/kg BW dose ($p<.001$). A group by drug interaction was observed at the 10mg/kg BW dose, with RU24969 diminishing PPI in wild-type mice but not in 5HT-1B-minus mice. Results suggest that activation of 5HT-1B receptors is responsible for startle amplitude reduction and deficits in PPI induced by RU24969 in mice. Further, activation of 5HT-1B receptors by endogenous serotonin may decrease PPI.

663.18

CHARACTERIZATION OF THE DISRUPTIONS OF PREPULSE INHIBITION AND HABITUATION OF STARTLE INDUCED BY α-ETHYLTRYPTAMINE. D.L. Martinez[1], M.A. Geyer[1,2], and V. Lehmann-Masten[2]*. Departments of Neuroscience[1] and Psychiatry[2], University of California San Diego, La Jolla, CA 92093-0804.

α-ethyltryptamine (AET), a monoamine oxidase inhibitor and potent monoamine releasing agent, is the first example of an indolealkylamine analog demonstrated to substitute in MDMA-trained animals. (Glennon, 1993). Previous studies have demonstrated that the substituted amphetamine/entactogen 3,4-methylenedioxymethamphetamine (MDMA) and AET have similar effects on unconditioned motor behavior in rats (Krebs and Geyer, 1993). Furthermore, the locomotor activating effects of both MDMA and AET are blocked by pretreatment with fluoxetine (Callaway and Geyer, 1990; Krebs and Geyer, 1993), a serotonin (5-HT) uptake inhibitor, suggesting that the two compounds may share a presynaptic mechanism of action. This study examined the effects of AET using measures of startle plasticity, specifically prepulse inhibition (PPI) and habituation. PPI, a measure of sensorimotor gating, is reduced in rats treated with hallucinogens, serotonin releasers, and dopamine agonists. In contrast, startle habituation is reduced in rats treated with hallucinogens and serotonin releasers. AET (2.5, 5.0 and 10.0 mg/kg) decreased PPI of acoustic startle and reduced the habituation of tactile startle. To determine whether AET produces these effects via pre- or post-synaptic actions, fluoxetine (2.5 and 10.0 mg/kg) was used as a pretreatment. Fluoxetine did not disrupt PPI, but did reduce startle habituation. Fluoxetine pretreatment prevented the AET-induced disruption of PPI, and reduced the AET-induced disruption of startle habituation. Combined with previous findings, these results confirm that the effects of AET on startle plasticity are due to the release of presynaptic 5-HT.

663.19

DISSOCIATION OF HIPPOCAMPAL SEROTONIN RELEASE AND LOCOMOTOR ACTIVITY FOLLOWING DRUG INJECTIONS INTO THE MEDIAN RAPHE NUCLEUS. I. Shim* and D.Wirtshafter. Dept. Psychology., Univ. Ill. at Chicago, Chicago, IL 60607-7137.

In vivo microdialysis was used to investigate the role of 5-HT in mediating the locomotor hyperactivity produced by injections of 8-OHDPAT, muscimol and baclofen into the median raphe nucleus (MR).

Intra-MR injections of muscimol produced a pronounced increase in locomotor activity and a concomitant decrease in hippocampal 5-HT release to 58% of baseline during the first hour post injection. 8-OHDPAT resulted in a much smaller effect on activity which was accompanied by a remarkable reduction of hippocampal 5-HT output to 49.1% of baseline. In addition, even though intra-MR injections of baclofen resulted in marked activity, the maximal magnitude of which was similar to that produced by muscimol, these injections were without effect on extracellular 5-HT release in the hippocampus. No injections altered 5-HIAA release.

These findings demonstrate that the effects of intra-MR injections of these drugs on activity and hippocampal 5-HT release can be clearly dissociated, and strongly suggest both that serotonin does not play an essential role in the production of the hyperactivity by muscimol or baclofen, and that the hyperactivity produced by intra-MR injections of different drugs is mediated through at least partially distinct cell populations within the MR.

663.20

5-HT_1, BUT NOT β-ADRENERGIC, ANTAGONISM, REDUCES THE EFFECTS OF A 5-HT_2 AGONIST K.M. Krebs* and M.A. Geyer Dept. of Psychiatry, UCSD, La Jolla, CA 92093-0804.

The existence of functional interactions between serotonin (5-HT) 1A and 2 receptors has been suggested. For example, chronic pretreatments with the 5-HT_{1A} agonist 8-OH-DPAT produced cross-tolerance to the effects of the selective 5-HT_2 agonist DOI on rat locomotor activity. The current experiments tested this putative interaction using the Behavioral Pattern Monitor (BPM), which measures amounts and patterns of locomotor and investigatory behavior. In a series of studies, male rats (n=10-11) received pretreatments of either vehicle or one of the nonselective 5-HT_1 and β-adrenergic antagonists, propranolol (10 mg/kg SC) or (+/-)pindolol (20 mg/kg SC) before receiving treatments of either saline or 1.0 mg/kg DOI (SC). Acutely, DOI reduced measures of locomotor activity. Both propranolol and pindolol pretreatment blocked the attenuation of locomotor activity produced by DOI. Thus, antagonism of either 5-HT_{1A}, 5-HT_{1B}, or β-adrenergic receptors, produced by propranolol and pindolol, decreases the expression of DOI-induced behavior. Because 5-HT_{1B} agonists increase locomotion, it is unlikely that the 5-HT_{1B} antagonist effects of propranolol or pindolol were responsible. To assess the relevance of β-adrenergic receptors to the effect of DOI, the selective β-adrenergic antagonist betaxolol was used as a pretreatment. Rats (n=10) received either saline or betaxolol (10 mg/kg SC) before treatments with saline or DOI (1.0 mg/kg SC). DOI again reduced locomotion, but this effect was insensitive to betaxolol, indicating that β-adrenergic antagonism was not responsible for the ability of pindolol and propranolol to block the effects of DOI. Thus, it is likely that propranolol and pindolol attenuated the effects of DOI on locomotor activity through 5-HT_{1A} antagonism. As DOI is reputed to be a selective 5-HT_2 agonist, this antagonism may reflect a functional interaction between 5-HT_{1A} and 5-HT_2 receptors in the expression of the effects of DOI on locomotor activity.

663.21

FLUOXETINE INCREASES STEADY-STATE LEVELS OF PREPROENKEPHALIN mRNA IN RAT STRIATUM AND AMYGDALA. P. Rossby, C. Perrin, A. Burt, I. Nalepa, S. Spector*, F. Sulser. Vanderbilt Univ., Sch. of Med., Nashville, TN 37232.

Fluoxetine and other selective serotonin reuptake inhibitors (SSRIs) are being prescribed extensively for a broad range of psychopathologies, including depression, obsessive-compulsive disorder, and post-traumatic stress disorder. Yet aside from the knowledge that SSRIs increase the synaptic availability of serotonin, little is known about the mechanism(s) of their actions in the CNS. We are therefore studying the effects of fluoxetine on the steady-state mRNA levels of genes putatively involved in the modulation of cognitive/emotional states. In this experiment we focused on the preproenkephalin (PPE) gene, which encodes two opiate peptides i.e., met- and leu-enkephalin. Male Sprague Dawley rats (250-300 g) were treated with fluoxetine (10 mg/kg, i.p.) or normal saline for 10 days. Total RNA was prepared from frozen tissues by the method of Chomczynski, and PPE mRNA levels were determined by quantitative slot blot or Northern blot analysis. The cDNA probe contained the entire rat PPE coding region. Chronic fluoxetine treatment induced a 2-fold increase in PPE mRNA in the amygdala and a 1.7- to 2-fold increase in the striatum versus saline treated controls. This is the first report implicating fluoxetine (and possibly serotonin) in the regulation of an opiate mRNA in vivo. (USPHS grant MH-29228).

663.22

5-HT1A AND OPIOID RECEPTORS HAVE DIFFERENT ROLES IN THE INHIBITION OF VOMITING. J.B. Lucot*. Dept. Pharmacology, Wright State Univ., Dayton, OH 45435

The 5-HT_{1A} receptors inhibitory to vomiting are postsynaptic, implying a physiologically relevant role in preventing vomiting. Endogenous opioids have been implicated in both eliciting and inhibiting vomiting. Antagonists were used to evaluate the role of these receptors in the regulation of vomiting elicited by motion in cats.

The dose of 1.0 mg/kg of the prototype 5-HT_{1A} antagonist LY 297996 completely reversed the antiemetic effect of the agonists 8-OH-DPAT, flesinoxan and LY 301317. Given alone, it had no effect on the number vomiting, the latency to the first retch, the duration of a bout of retch/vomits or the number of multiple bouts of retch/vomits. The highest dose of naloxone tested, 0.1 mg/kg (1/100th the emetic dose) did not alter the latency to the first retch but did increase the incidence and the number with multiple bouts and significantly increased the duration of the first bout of retch/vomits. These results suggest that stimulation of 5-HT_{1A} receptors decreases the probability of vomiting while endogenous opioids end a vomiting sequence and produce a relatively refractory period.

663.23

SEROTONERGIC MODULATION OF AVIAN COMPULSIVE PECKING. L.J. Goodman*, P.D. Skosnik, L.J. Beeler & C.F. Gaskill. Dept. of Psychology, West Virginia Univ., Morgantown, WV 26506.

Compulsive pecking is a stereotyped, species-specific behavior that may be induced in birds by a variety of stimuli. DAergic mechanisms of this behavior have been previously established in pigeons. Compulsive behaviors in mammals have also implicated serotonin, as in humans (e.g., in OCD patients) where a serotonin reuptake inhibitor (e.g., clomipramine (CLO) reduces compulsive behavior. The present study assessed and compared acute and chronic effects of CLO on apomorphine (APO) induced pecking (AIP) and restricted feeding induced pecking (FIP) in Columba livia..

Under FIP conditions, acute testing of pre-feeding injection of CLO demonstrated a negative dose (5-15 mg/kg, ip)-FIP response relationship, the higher the CLO dose the greater the FIP suppression compared to saline injection. TFMPP (2 mg/kg, ip), another 5-HT agonist, also acutely reduced FIP. Chronic CLO injections (21 days, 15 mg/kg/day), aside from a first day effect, did not produce a sustained reduction in FIP until day 19. Reduced FIP was often accompanied by marked pacing. Seven days of saline were required for recovery to elevated pre-chronic CLO FIP. Under AIP testing, a first day reduction in pecking occurred with CLO but acute TFMPP produced no change from APO alone. Chronic CLO (24 days), unlike in FIP, did not alter AIP scores.

Differential results from such assessments may provide useful insight into interactive mechanisms of DA and 5-HT systems in stereotypy.

663.24

SEROTONIN INNERVATION OF DOPAMINE NEURONS IN RAT VENTRAL TEGMENTUM. C.F. Phelix[1], M.J. Russell[1], P. Kumar*[1], P.A. Broderick[2] 1 - Div. Life Sci., Univ. Texas, San Antonio, TX 78249-0662; 2 - Dept. Pharm., CUNY Medical School, New York, NY 10031

There are two sights of presynaptic interaction proposed for serotonin (5HT) to affect tegmental dopamine (DA) neurons, i.e., proximal and distal. One sight of distal interaction is at the convergence of their terminals, e.g., nucleus accumbens (NAcc; Phelix and Broderick, Brain Res. Bull. 37:37-40, 1995). Our present objective was to determine the degree of overlap between 5HT axons and DA somatodendrites in the ventral tegmental area (VTA) and substantia nigra, the proximal sights. In addition, tract tracing studies were performed to identify mesoaccumbens DA neurons innervated by 5HT axons. The first part of this study utilized a double label light microscopic immunocytochemical procedure to stain for 5HT first with silver-diaminobenzidine (DAB) and second for tyrosine hydroxylase (TH) with DAB alone. With this approach 5HT axons were seen to inundate DA neurons forming axodendritic and axosomatic contacts. These contacts extended from DA neuron populations in the midline VTA to the dorsolateral substantia nigra pars compacta. The second part of this study included retrograde transport of horse radish peroxidase from the ventrolateral NAcc to the VTA before staining with 5HT, that is both were visualized with silver intensified DAB. Then TH was visualized with DAB. Most mesoaccumbens DA neurons received axodendritic and axosomatic 5HT innervation. These results show the pervasive character of the presynaptic influence of 5HT on DA neurons within reward and motor circuits. Supported by HHMI and HL02914-01 to CFP.

663.25

CORTICOSTERONE DETERMINES SPECIFIC 5-HT$_{1A}$ RECEPTOR-MEDIATED RESPONSES IN A WATER MAZE LEARNING TASK.
O.C. Meijer*, R. Kortekaas, M.S. Oitzl and E.R. de Kloet. Div. of Medical Pharmacology, LACDR, P.O.B. 9503, 2300 RA Leiden, The Netherlands

We have tested the effects of the specific 5-HT$_{1A}$ receptor agonist 8-OH-DPAT in animals with different plasma levels of corticosterone (B) in the free swim trial of the Morris water maze. Sham operated male Wistar rats with normal circadian rhythmicity were compared to rats with defined exogenous levels of B. Rats were adrenalectomized and implanted with a 20% corticosterone pellet, to obtain constant low levels of circulating B. One week after surgery the animals were trained for 4 days to find a submerged platform in a pool. On the 5th day the platform was removed and the behaviour of the animals was monitored during a 1-minute swim session. 90 Min before this free swim trial an s.c. injection of 1 mg/kg B (High B) or vehicle (Low B) was given; 30 min before the trial 0 or 100 μg/kg 8-OH-DPAT was injected i.p.

8-OH-DPAT had a stimulatory effect on the distance swum by the animals of all 3 groups. There were no effects of 8-OH-DPAT on initial memory retrieval, measured as the distance swum upto the first crossing of the previous platform location. As a measure for search strategy in the free swim trial the number of crossings of the previous platform location was used. Rats with high B, but no 8-OH-DPAT had significantly more crossings than sham or Low B rats. In response to 8-OH-DPAT, both sham and High B rats showed a decrease in the number of crossings. Low B rats were completely unresponsive to the effect of 8-OH-DPAT on this parameter.

These results show two different effects of B on the behaviour of rats in the free swim trial of the Morris water maze: 1) High B per se increases use of the knowledge of the previous platform location compared to sham and Low B rats. 2) The 5-HT$_{1A}$ receptor-mediated response to 8-OH-DPAT in the hippocampus is blocked by low levels of B; high levels of B can override this effect. This last point provides a striking parallel between the hormonal dependency of the activity of single CA1 neurons and a parameter in a spatial memory task in response to 5-HT$_{1A}$ receptor stimulation by 8-OH-DPAT.

NEUROPEPTIDES AND BEHAVIOR II

664.1

DIFFERENTIAL REGULATION OF VASOPRESSIN RECEPTORS IN THE HYPOTHALAMUS OF GOLDEN HAMSTERS.
Y. Delville* and C.F. Ferris. Behavioral Neuroscience Program, Psychiatry Dept., Univ. of Mass. Med. Ctr., 55 Lake Ave. N., Worcester, MA 01655.

In female golden hamsters, ovariectomy results in a loss of vasopressin (AVP) binding from the ventrolateral hypothalamus (VLH). It is likely that periods of the reproductive cycle associated with low plasma levels of gonadal steroids, such as lactation, coincide with low density of AVP receptor binding within the VLH. This possibility was tested by comparing the density of AVP receptor binding in the VLH of lactating and cycling females. As predicted, lactation was associated with a total loss of AVP receptor binding from the VLH. Interestingly, lactation was also associated with a dramatic increase in AVP receptor binding density within another hypothalamic site, the dorsomedial nucleus (DMN). Our data support the hypothesis AVP receptor binding within the VLH is dependent upon gonadal steroids. In contrast, AVP receptor binding within the DMN may be responsive to other neurobiological changes associated with lactation. The function of increased AVP receptor binding within the DMN during lactation is currently being tested in the context of behavioral modifications associated with lactation. (Supported by NSF BNS-9121097 awarded to C.F.F.)

664.2

BEHAVIORAL AND NEUROBIOLOGICAL CONSEQUENCES OF ADOLESCENT ABUSE IN GOLDEN HAMSTERS.
C.F. Ferris*, R.H. Melloni, M.A. Abbott and Y. Delville. Behavioral Neuroscience Program, Psychiatry Dept., Univ. Mass Med. Ctr., 55 Lake Ave N., Worcester, MA 01655.

Physical and emotional abuse in human adolescence is associated with changes in context dependent aggression. We hypothesize that environmental and emotional insult during this developmental period have behavioral and neurobiological consequences into adulthood. In golden hamsters, a similar period to adolescence can be identified in sub adult animals. Between postnatal days P28-P42, sub-adult hamsters were subjected to daily attacks and threats by aggressive adults for 30 min periods. Following the cessation of abuse, animals were tested for context-dependent aggression as young adults on P45 in a resident/intruder model. When exposed to younger and smaller males placed into their home cage, abused animals were significantly more aggressive than controls. However, when confronted by animals of equal age and size, abused hamsters were submissive. Since vasopressin and serotonin are involved in the control of aggression, brains are being analyzed for changes in these neurotransmitters. (Supported by NSF BNS-9121097)

664.3

VASOPRESSIN/SEROTONIN INTERACTIONS IN THE ANTERIOR HYPOTHALAMUS CONTROL AGGRESSIVE BEHAVIOR IN GOLDEN HAMSTERS.
R.H. Melloni, Jr.*, Y. Delville, and C.F. Ferris, Behavioral Neuroscience Program, Psychiatry Dept., University of Massachusetts Medical Center, Worcester, MA 01655

This study examines the specific anatomic and pharmacologic nature of the AVP/5-HT neural systems in the anterior hypothalamus (AH) of golden hamsters, their involvement in the control of aggressive behavior, and their interactions with fluoxetine, a 5-HT uptake inhibitor shown to decrease aggressive behavior in many species. We hypothesized that AVP facilitates aggression by enhancing the activity of AH neural network controlling offensive aggression that is normally restrained by 5-HT. To test this hypothesis, we first employed receptor binding and immunohistochemistry to examine whether AVP and 5-HT and their receptors were colocalized in the AH. These studies showed high density of both AVP and 5-HT receptor binding in this brain region and revealed the presence of putative 5-HT synapses on AVP neurons in particular regions of the AH. Next, we tested whether AVP microinjections in the AH could stimulate offensive aggression, and if this behavior could be selectively inhibited by 5-HT or fluoxetine. AVP microinjection in the AH resulted in a marked increase in offensive aggression that could be blocked by the co-injection of 5-HT or the peripheral administration of fluoxetine. Finally, *in vivo* microdialysis was used to measure directly AVP and 5-HT levels in the AH following fluoxetine treatment. Fluoxetine treatment caused an approximate 4-fold increase in 5-HT levels in the AH, and a coincident with a 3-fold decrease in AVP levels. In summary, we provide anatomical, behavioral and pharmacological evidence supporting a functional relationship between the AVP/5-HT neural systems in the AH and the control offensive aggression.

664.4

IBOTENIC LESIONS OF CENTRAL AMYGDALA INHIBITS VASOPRESSIN-INDUCED FLANK MARKING.
M. Bamshad* and H.E. Albers. Lab. Neuroendocrinol & Behav., Depts. Biol. & Psychol., Georgia State Univ., Atlanta, GA 30303.

Syrian hamsters communicate social information by a form of scent marking called flank marking. Flank marking can be stimulated by microinjection of arginine vasopressin (AVP) into either the medial preoptic-anterior hypothalamus continuum (MPOA-AH), the lateral septum-bed nucleus of stria terminalis (LS-BNST) or the periaqueductal gray (PAG). Previously, we reported increases in Fos-like immunoreactivity in the BNST, PAG and central amygdala (Ce) of hamsters microinjected with AVP into the MPOA-AH. To investigate whether the Ce is involved in AVP-induced flank marking the Ce of hamsters was lesioned with ibotenic acid. Hamsters were injected bilaterally with either saline or ibotenic acid in the Ce and then implanted with a guide cannula aimed at the MPOA-AH. A week later, hamsters were microinjected with AVP into the MPOA-AH and tested for flank marking. The frequency of flank marking was significantly reduced in hamsters with Ce lesions ($p<0.05$). The ibotenic acid injected animals flank marked 4.8 ± 3.60 during the 10 min. test whereas the saline injected animals marked 36.6 ± 11.37. These data suggest that signals from the MPOA-AH to the Ce are important for flank marking and that Ce is yet another component of the neural circuit that regulates AVP-induced flank marking. (Supported by NSF IBN 9222099).

664.5

FLANK MARKING STIMULATED BY VASOPRESSIN (AVP) INJECTED INTO THE MEDIAL PREOPTIC AREA-ANTERIOR HYPOTHALAMUS (MPOA-AH) IS INHIBITED BY AVP AND NMDA ANTAGONISTS INJECTED INTO THE PERIAQUEDUCTAL GRAY (PAG). T.T.Cooper*, M. Bamshad, and H.E. Albers. Laboratory of Neuroendocrinology and Behavior, Depts. of Biology & Psychology, Georgia State University, Atlanta, GA 30303

The MPOA-AH and PAG are components of the neural circuit controlling flank marking in Syrian hamsters. Microinjection of AVP into the MPOA-AH or PAG elicits high levels of flank marking and microinjection of AVP combined with a NMDA antagonist into the MPOA-AH inhibits flank marking. The purpose of the present study was to examine the effects of an AVP antagonist (AVP-A) and a Glu antagonist (AP5) microinjected into the PAG on flank marking stimulated by AVP microinjection into the MPOA-AH. Hamsters (n=8) were injected with either AVP-A (1.0mM), AP5 (1.0mM), metergoline (5-HTa, 10.0μM), or saline into the PAG followed by microinjection of AVP (9.0μM) into the MPOA-AH (counterbalanced across test days). Hamsters exhibited high levels of flank marking following injection of saline (27.38$\pm$4.82) or 5-HTa (19.63$\pm$5.20) into the PAG, but exhibited significantly ($p<.01$) lower levels of flank marking when either AVPA (6.75$\pm$6.06) or AP5 (6.88$\pm$2.89) was injected into the PAG. These data indicate that activation of both AVP and NMDA receptors in the PAG are important for eliciting flank marking following microinjection of AVP into the MPOA-AH.

(Supported by NSF IBN-9222099)

664.6

FOS-LIKE IMMUNOREACTIVITY ASSOCIATED WITH MATING-INDUCED AGGRESSION IN MALE PRAIRIE VOLES (*MICROTUS OCHROGASTER*). T.J. Hulihan, Z.X. Wang* and T.R. Insel. Department of Psychiatry and Behav. Sci., Emory Univ., Atlanta, GA 30322, USA

We have been studying monogamous prairie voles (*Microtus ochrogaster)* to investigate the neural substrates of pair bonding. After mating, male prairie voles develop a pair bond with selective affiliation and aggression. Selective aggression is defined as attack of a novel conspecific while defending the mate. This behavior is associated with monogamy (non-monogamous voles do not show selective aggression) and appears to be induced by mating (cohabitation with a female does not induce aggression). In this study, we examined regional brain Fos induction associated with selective aggression. In Experiment 1, male prairie voles that mated with a female for 24 hrs (MT) showed aggression in a resident-intruder test (RI; Winslow et al., 1993), as well as increased Fos-like immunoreactivity (Fos-ir) in the lateral septum (LS), the bed nucleus of the stria terminalis (BST), medial amygdaloid nucleus (MA), and the ventromedial hypothalamus (VMH), in comparison to the mated voles without RI test. In Experiment 2, MT males showed more aggression than the males that cohabited with a female for 24 hrs without mating (CH) or that had no exposure to a female (NF). MT males also had increased Fos-ir staining in the accessory olfactory bulb (AOB), dorsomedial hypothalamus (DMH), and MA than CH and NF males, and the males that had neither exposure to a female nor RI test (control). In addition, MT and CH males had increased Fos-ir staining in the LS and BST than NF and control males. In Experiment 3, MT males showed more aggression and Fos-ir staining when tested by a male intruder than by a female intruder or a female partner. These data indicate distinct brain areas that may be involved in the induction or regulation of mating-induced selective aggression in male prairie voles.

664.7

Enkephalinergic Immunoreactivity is Distributed throughout the Social Behavior Pathways of the Male Syrian Hamster Brain. A. G. Holt* and S. W. Newman. Neuroscience Program and Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109-0616

The objectives of this study were (1) to determine whether methionine enkephalin (met-E) and/or leucine enkephalin (leu-E) are distributed within neural pathways implicated in mating and aggression in the male Syrian Hamster and (2) to compare and contrast the distribution of met-E and leu-E immunoreactivity (ir). Twenty-two behaviorally naive male hamsters received 50-360μg of colchicine, a compound which disrupts axonal transport, administered intracerebroventricularly under anesthesia 48 hours prior to perfusion. Hamsters were perfused transcardially with 4% paraformaldehyde and 40μm brain sections were processed for met-E or leu-E ir using one of several polyclonal antibodies, rabbit anti-met-E or anti-Leu-E antibody from Incstar Inc., or one of several rabbit anti-met-E antibodies from Dr. S.J. Watson, at a dilution of 1:1000. The ABC method (Vectastain Elite) was utilized with NiCl enhanced DAB. To determine the specificity of the primary antibodies, preabsorption and cross-reactivity studies were done with met-E, leu-E and other peptides. The olfactory bulbs (OB), preoptic area, and bed nucleus of the stria terminalis (BNST), regions that play a role in copulatory behavior, contained both met-E and leu-E ir as did regions implicated in aggressive behavior, including the central amygdaloid nucleus, paraventricular nucleus of the hypothalamus, arcuate nucleus, periaqueductal gray, and ventromedial nucleus of the hypothalamus. The distribution pattern of met-E and leu-E ir differed from that of dynorphin and beta endorphin reported previously. The results of the preabsorption and cross-reactivity control studies suggested that the specificity of the leu-E antibody was less than that of the met-E antibodies. The distribution of met-E and leu-E ir overlapped in all areas studied with the exception of the OB and BNST, where only met-E or Leu-E ir, respectively, was found. The quality and amount of immunostaining was greater with the met-E antibodies than with the leu-E antibody. (Supported by NIH, GM13553 and NS20629)

664.8

BEHAVIORAL EFFECTS OF INTRACEREBROVENTRICULAR INJECTION OF SELECTIVE TACHYKININ AGONISTS AND ANTAGONISTS. **T.C.M. de Lima*, R.M. Teixeira, A.R.S. Santos, G.A. Rae and J.B. Calixto. Depto. de Farmacologia, CCB, UFSC, Florianópolis, SC, 88040-900, Brazil.**

Tachykinins promote several biological effects by activation of NK_1, NK_2 and NK_3 receptors (Maggi et al., 1993, J.Auton.Pharmacol. 13:23-93). Stimulation of NK_1 and NK_2 receptors induces an anxiolytic behavior in the mouse light-dark box (Zernig et al., 1992, Neurosci.Lett. 143:169-172; Stratton et al., Eur.J.Pharmacol. 250:R11-R12). In the present study mice were injected (5 µl) intracerebroventricularly with 0.1, 1, 10, 100 or 500 pmol of substance P (SP), SP methyl ester (SPME), [ß-Ala^8] neurokinin A_{4-10} (ßALA), senktide (SENK), NK_1, NK_2 and NK_3 receptor agonists, respectively; FK888 (FK) or SR48968 (SR), NK_1 and NK_2 receptor antagonists, or vehicle (PBS). Immediately after drug injection they were placed in an open-field for habituation. After 5 min they were tested on an elevated plus-maze (EPM) for an additional 5 min session. SP did not modify any of EPM parameters, but SPME caused a reduction in frequency of open arm (OAE) and a decrease in enclosed arm (EAE) entries ($p<0.05$). ßALA just increased EAE in the highest dose, while SENK enhanced OAE and percent open arm time (OAT; $p<0.05$). FK increased OAT and SR enhanced OAE and OAT ($P<0.05$). None of these compounds changed percent enclosed arm time or motor coordination in a rota rod test. These results suggest that tachykinins play a modulator role in anxiety evaluated on EPM test in mice : NK_1 and NK_2 receptors activation inducing an anxiogenic whereas NK_3 stimulation producing an anxiolytic action.

Supported by CAPES and CNPq.

664.9

TREATMENT WITH SUBSTANCE P IN AN ANIMAL MODEL OF HEMI-PARKINSONISM: INDICES FOR PROTECTIVE AND RECOVERY-PROMOTING EFFECTS

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The neuropeptide substance P (SP) is known to act as a neurotransmitter or neuromodulator in various parts of the nervous system. Apart from its enhancing effects on reinforcement and memory, found after central and peripheral administration, there is evidence that SP can also have neurotrophic effects. Since SP is anatomically and functionally related with the nigrostriatal dopamine (DA) system, such neurotrophic effects of SP may play a role in cases of experimental damage of DA neurons, and in relevant neurodegenerative disorders, especially in Parkinson's disease. Thus, in a previous experiment (Mattioli et al., Neuroscience 48'92, 595-605), we administered SP daily (50μg/kg, ip) starting with the day after unilateral 6-OHDA injection into the rat substantia nigra and found that such post-lesion treatment with SP not only promoted recovery from the lesion-dependent deficit in thigmotactic scanning, but also prevented the ipsiversive asymmetry in turning in animals with subtotal depletions of neostriatal DA. These results indicated that SP might have acted also in a protective way. Thus, a subsequent experiment was conducted where rats received the unilateral 6-OHDA injection after 1 week of daily treatment with SP (50μg/kg, ip). An additional group was treated with cholecystokinin (CCK, 1μg/kg, ip), another neuropeptide also closely associated to DA in the forebrain. The analysis of behavioral asymmetries during 2 weeks after 6-OHDA injection showed that animals with pre-lesion SP treatment showed less behavioral asymmetry than vehicle- or non-treated controls, whereas the asymmetries were even stronger in CCK-treated animals. Thus, these results strengthen our hypothesis that SP can act in a protective way.

664.10

BEHAVIORAL EFFECT OF THE NK 2 ANTAGONIST SR 48968 BUT NOT OF THE NK 1 ANTAGONIST SR 140333 IN THE MOUSE BLACK AND WHITE BOX MODEL. G. Bematzky*, A. Saria *Central Animal Facility, Univ. of Salzburg, Austria; Neurochemical Laboratory, Univ. of Innsbruck, Austria

Previous reports have suggested the envolvement of neurokinin (NK) receptors in anxiety as tested in the black and white box (BWB) behavioral paradigm. We studied the new antagonists of NK1, SR 140333 and of NK 2, SR 48968 in the BWB. Swiss albino mice were kept in groups of 10 - 12 mice under conventional housing conditions and controlled reversed lighting. Diazepam, SR 140333 and SR 48968 or vehicle were injected ip30 min (10 ml/kg) before testing in the BWB. The experimenter was blind to the treatment until after the evaluation of the videotapes. Briefly, mice were placed in the center of the brightly lit (60 W white light) compartment of a two-compartment high-walled open-topped box. Like diazepam, the NK 2 antagonist SR 48968 increased time in the white, crossings in the white and rearings in the white, suggesting an anxiolytic effect. The NK 1 antagonist SR 140333 did not change the time spent in the light or dark compartment. The observed increase of crossings in the white and rearings in the white as well as the increase of the same behavioral parameter in the dark compartment, suggest higher activity of these animals compared with the solvent controls. However since also the number of transitions was higher than in the controls and comparable with different solvent groups we believe that such differences may result from normal variations in basic activity of this strain of mice. It is concluded that blockade of NK 2 receptors in mouse brain leads to an effect comparable with that of anxiolytic drugs. No NK 1 specific behavioral effect could be detected in the black and white box paradigm. (This study was supported by the Mundipharma Company.)

664.11

Neuropeptide Y (NPY) and D-Trp^{32}-NPY increase monoaminergic neurotransmission in rat hypothalamic dialysates during feeding behavior. F. F. Matos*, V. Guss and C. Urban. CNS Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford CT, USA

Administration of NPY into the hypothalamus or ventricles induces marked increases in food consumption in satiated rats. It has been suggested that the brain monoamines are involved in NPY-induced feeding. This study investigated the effects of NPY and the proposed NPY antagonist, D-Trp^{32}-NPY, on extracellular norepinephrine (NE), dopamine (DA), serotonin (5-HT), and metabolites 5-HIAA, DOPAC and HVA in the rat hypothalamus. Microdialysis probes were implanted in the vicinity of the periventricular hypothalamic nuclei (PVN) and a guide cannula in the lateral ventricle for intracerebroventricular (icv) administration of NPY and D-Trp^{32}-NPY. Dialysate levels of NE, DA, 5-HT, 5-HIAA, DOPAC and HVA were determined by HPLC-ECD. Feeding behavior was measured for 2 hours while animals were continuously dialyzed. NPY (20 µg, icv) significantly increased extracellular levels of NE (1.5 fold), DA (2.5 fold), DOPAC (1.5 fold) and HVA (3 fold), but did not change 5-HT or 5-HIAA levels. While NPY increased neurotransmitters and metabolites, food intake increased to 3.5±0.3 g (n=26). Food intake in control animals was 0.13±0.05 g (n=22). The putative NPY antagonist, D-Trp^{32}-NPY (40 µg, icv) produced similar increases in extracellular levels of NE (1.7 fold), DA (2.5 fold), DOPAC (3.5 fold) and HVA (2 fold) as NPY but did not change 5-HT or 5-HIAA levels. D-Trp^{32}-NPY also significantly increased food intake to 1.8±0.4 g (n=10). Administration of D-Trp^{32}-NPY (40 µg, icv) 5 min before NPY did not significantly change the increases in NE and DA produced by NPY alone. In contrast, D-Trp^{32}-NPY attenuated the later increase in DOPAC and HVA induced by NPY. In these animals, food intake increased to 3.7±1.2 g (n=7). These data indicate that activation of the hypothalamic monoaminergic system is involved in NPY-induced feeding and that D-Trp^{32}-NPY is a partial agonist at NPY receptors.

664.12

THE ROLE OF ANGIOTENSIN II IN THE MOTIVATIONAL AND PREFERENTIAL FACTORS OF DRINKING. L. Stubley-Weatherly*, J.N. Weatherly, and J.W. Wright. Dept. of Psychology, Washington State University, Pullman, WA 99164-4820

While it is well established that an intracerebroventricular (icv) infusion of Angiotensin II (AngII) induces a drinking response in an organism, the functional motivation underlying this drinking is unknown. The present study examined an animal's preference for drinking different liquids (H_2O versus a 16% sucrose solution) over a 24 hour period following an infusion of either AngII or aCSF. Male Sprague-Dawley rats were implanted with an icv cannula through which AngII (100 pmol in 2 ul aCSF) or aCSF was delivered. After an infusion, an animal's fluid intake of either H_2O, 16% H_2O/sucrose solution, or H_2O and 16% H_2O/sucrose solution was measured. Thus, factors involved in AngII-induced drinking, such as whether drinking was motivated by a biological need for a fluid or whether fluid preference controlled fluid intake, could be assessed. Furthermore, monitoring drinking over a 24 hour period allowed for the assessment of any interaction between these variables. Preliminary data suggests that an interaction exists between total fluid intake and fluid preference. Although AngII infusions mediate simple generalized drinking responses, it also appears that other motivational factors still exert control over an animal's drinking behavior.

664.13

DEVAZEPIDE, A CCK_A RECEPTOR ANTAGONIST, BLOCKS THE ACQUISITION OF CONDITIONED REWARD. F.J. Vaccarino [1], P. Li[1]*, V.P. Franco and S.A. Josselyn, **Dept. of Psychology and Psychiatry[1], University of Toronto, Toronto, Canada M5S 1A1.**

Cholecystokinin (CCK) is co-localized with dopamine (DA) in portions of the mesolimbic system, where it may facilitate the function of DA through the CCK_A receptor subtype. DA has been implicated in the acquisition of conditioned reward (CR), raising the possibility of a role for endogenous CCK in this process. Exp. 1 examined the effects of systemic administration of the CCK_A receptor selective antagonist, devazepide (0, 0.001, 0.01, 0.1 mg/kg, ip) on the acquisition of CR. Two novel levers were presented to drug-free animals in a test session; depression of the CR lever produced a light-tone stimulus previously paired with food delivery while depression of the non-CR lever produced no programmed consequence. Animals receiving vehicle treatments prior to the food-CS conditioned sessions responded more frequently on the CR lever during the test session. However, pre-treatment with devazepide (0.1 but not 0, 0.001, 0.01 mg/kg) in the conditioning sessions blocked the acquisition of CR. The possibilities that devazepide (0.1 mg/kg) impaired the development of CR by decreasing the amount of food consumed or by inducing a conditioned taste aversion to the food were ruled out in Exps. 2 and 3. Together, these results suggest that intact CCK_A function may be necessary for the development of CR.

664.14

INTRA-AMYGDALA INFUSION OF PENTAGASTRIN, A CCK_B AGONIST, PRODUCES A FACILITATION OF THE ACOUSTIC STARTLE REFLEX. S. A. Josselyn*, P. W. Frankland, F. J. Vaccarino, and J. S. Yeomans, **Dept. of Psychology, University of Toronto, Toronto, Canada M5S 1A1.**

The startle reflex is enhanced by both conditioned and unconditioned "fear". The amygdala appears to play a critical role in the mediation of these effects since lesions of various amygdala nuclei abolish increased startle responding, while electrical stimulation of the amygdala and its output pathways increase startle amplitudes (e.g., Davis, 1992). Several lines of evidence suggest that activation of cholecystokinin$_B$ (CCK_B) receptors produces anxiety and panic in laboratory animals and humans. CCK is found in the basolateral nucleus of the amygdala. Systemic administration of CCK-4 or pentagastrin, which are selective for the CCK_B receptor, increases anxiety-like behaviors measured in a number of different behavioral paradigms. Here we test whether the central infusion of CCK, either intracerebroventricularly (icv) or into the amygdala, increases startle amplitudes. Following icv infusion of the CCK_B agonist pentagastrin (0, 1.0, 10.0 nM in 5.0 µl), we recorded startle amplitudes for a period of 2 hours. No increases in startle amplitudes occurred over this period compared to pre-infusion baseline levels. In contrast, infusion of pentagastrin into the amygdala (0, 0.01, 0.1, 1.0, 10.0 nM in 0.5 µl) produced large increases in startle amplitudes at the three highest doses compared to pre-infusion baselines. These increases occurred 5-10 minutes following the drug infusion, and persisted for the remainder of the 30-minute test.

664.15

THE EFFECT OF CORTICOTROPIN RELEASING HORMONE ON THE ACOUSTIC STARTLE REFLEX. S.G. Birnbaum, M.S. Lidow* and M. Davis. Dept. of Psychiatry and Section of Neurobiology*, Yale Univ. Sch. Med., New Haven, CT 06508

Stimulation and lesion studies have implicated the amygdala in modulation of the acoustic startle reflex. The amygdala projects directly to the nucleus reticularis pontis caudalis (PnC), an obligatory synapse in the acoustic startle pathway, and contains a high level of corticotropin releasing hormone (CRH) neurons. The present study evaluated the effect of local infusion of CRH into the PnC on startle amplitude .

Eighteen rats were implanted with a cannula unilaterally in the PnC. One week following surgery all rats received a 5-min acclimation period followed by 60 startle stimuli (105-dB bursts of white noise, 50-msec duration, 30-sec interstimulus interval) to establish a stable baseline. They were then removed from the startle chamber and infused with artificial cerebral spinal fluid (ACSF) or CRH (10, 20, and 40 ng) according to a Latin square design. They were immediately replaced into the startle chambers and tested with 120 startle stimuli. Testing occurred every other day until each rat had received all doses. Two days following the last test session, all animals received the same testing procedure with either ACSF or a 80-ng CRH dose in a cross-over design.

CRH produced a dose-dependent increase in the startle amplitude which was significant at all doses. This effect began immediately after infusion and peaked at approximately 25 min. The facilitation of startle amplitude persisted for the 60-min test session. These results suggest that modulation of startle amplitude may occur by CRH release at the level of the PnC, perhaps following activation of the amygdala.

664.16

ROLE OF THE BED NUCLEUS OF THE STRIA TERMINALIS AND THE AMYGDALA IN THE EXCITATORY EFFECT OF CRH ON THE ACOUSTIC STARTLE REFLEX. Y. Lee* and M. Davis. Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508.

Corticotropin releasing hormone (CRH) is known to markedly increase the amplitude of the acoustic startle reflex after intraventricular (i.c.v.) infusion (CRH-enhanced startle; Swerdlow et. al. '86, Liang et. al. '92). Although intra-amygdala infusion of CRH did not mimic CRH-enhanced startle, we found that large electrolytic lesions of the amygdala complex blocked this effect. This suggested that the amygdala may play an obligatory role in the expression of this effect but may not be the primary receptor site for CRH given i.c.v. Subsequently, we reported that electrolytic lesions of the medial septum, but not the lateral septum, blocked CRH-enhanced startle, and intra-medial septum injection of CRH induced a small but significant enhancement of startle amplitude (Lee & Davis, '93). However, kainic acid lesions of the medial septum failed to block CRH-enhanced startle, which suggested that the blockade of CRH-enhanced startle by electrolytic lesions of the medial septum was due to damage of fibers of passage, presumably the fornix. In the present studies, we investigated the role of the bed nucleus of the stria terminalis (BNST) and the sub-nuclei of the amygdala in CRH-enhanced startle.

Electrolytic lesions, as well as NMDA lesions, of the BNST blocked CRH-enhanced startle. However, neither ibotenic acid lesions of the central nucleus nor NMDA lesions of the basolateral nucleus of the amygdala appeared to block CRH-enhanced startle, even though both amygdala lesions completely blocked fear-potentiated startle, another behavioral measurement sensitive to amygdala manipulation. These data suggest that the blockade seen with electrolytic lesions of the amygdala complex was due to damage of fibers of passage, perhaps afferent or efferent fibers of the BNST.

At the present time, it is not clear whether the BNST is the primary receptor site which mediates CRH-enhanced startle, or whether it is an important relay station for expressing this effect. Currently we are testing both possibilities by infusing CRH directly into the BNST and by investigating the role of various other structures which are close to the ventricles and project to the BNST via the fornix, such as the ventral hippocampus, in CRH-enhanced startle.

665.1

BEHAVIORAL PROFILE OF PUTATIVE ANTICRAVING DRUGS IN AA RATS, AN ANIMAL MODEL OF ALCOHOLISM. J. De Vry, R. de Beun and T. Glaser*. Institute for Neurobiology, Troponwerke, Berliner Strasse 156, D-51063, FRG

Alcohol preferring AA rats, selectively bred for a high daily consumption of ethanol (EtOH, > 5 g/kg, 10 % V/V) and preference (> 70 %) for EtOH over water, were tested with a variety of putative anticraving drugs, injected shortly before the 12 h test session. Absolute and relative (preference) EtOH intake, as well as total fluid and food consumption were assessed. In general, drugs could be categorized according to the particular profile of drug effect. One typical profile was obtained with (cis)-flupenthixol, with a clear suppression of consummatory behavior (both food and fluid). Although EtOH intake was reduced by this compound, the effect was completely nonselective (consequently, EtOH preference was not affected). A different profile was obtained with imipramine and fenfluramine, as these compounds reduced both EtOH intake *and* preference. However, both compounds also reduced food intake and this effect was already present at doses below the doses which affected EtOH intake. The largest group contained compounds which were able to reduce EtOH intake and preference, but this effect occurred at similar doses which suppressed food intake (fluoxetine, lisuride, nifedipine, nitrendipine, verapamil, buspirone). In the case of 8-OH-DPAT and nimodipine, there appeared to be a slightly more selective profile (i.e., small ratio between EtOH and food intake reduction). The most selective profile was obtained by diazepam and ipsapirone. In both cases, clear reduction of EtOH intake and preference was obtained at doses which did not suppress food intake. The last group (ritanserin, ondansetron, diltiazem and naltrexone) consisted of compounds which were without effect on any parameter. These data suggest that the AA rat model offers the opportunity to find drugs which suppress EtOH intake and preference in a selective manner, but it remains unclear to what extent such profile reflects specific anticraving properties.

665.2

INTRAVENOUS HEROIN AND ALCOHOL SELF-ADMINISTRATION BY ALCOHOL-PREFERRING AA AND ALCOHOL-AVOIDING ANA RATS. P.Hyytiä*, G. Schulteis and G.F. Koob. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The AA (Alko, Alcohol) rat line, selected for high alcohol drinking, has previously been shown to drink more solution containing the opiate etonitazene than its counterpart, the alcohol non-preferring ANA (Alko, Non-Alcohol) rat line (Hyytiä and Sinclair, *Psychopharmacology* 111:409,1993). The present experiments were conducted to see whether the relationship between ethanol and opiate intake by these lines would persist if an intravenous route of administration is used. Naive AA and ANA rats (n=6 per line) were trained to lever press using food as a reinforcer. Rats were then implanted with a silastic catheter into the external jugular vein. After recovery, animals were trained to respond for a 0.1 ml i.v. infusion of heroin (0.03 mg/kg/infusion) on a fixed-ratio (FR) 1 schedule with a 20-sec timeout (TO) during 3-h sessions. Once stable baselines were achieved, rats were given 3 within-session dose-response sessions during which rats were allowed to respond for ascending heroin doses (0.0075, 0.015, 0.03, and 0.06 mg/kg). The ratio requirement was then gradually increased to FR5 and the subjects were tested during one 6-h progressive-ratio session. There were no significant differences between AA and ANA rats either during the baseline heroin sessions, across the ascending within-session doses, or on the progressive-ratio probe. However, when after additional heroin baseline sessions, the rats were given ethanol intravenously (1.0 mg/kg/infusion) substituted for heroin on an FR1 TO 10-sec schedule, AA rats initially increased their responding, while ANAs fell below their heroin baseline. AA rats maintained stable responding and were significantly higher than ANAs also at higher ethanol doses, 2.0 and 4.0 mg/kg. These findings do not give evidence for a line difference in i.v. opiate self-administration but suggest that at least some factors contributing to the line difference in alcohol drinking are present when a non-oral route of administration is used. Supported by grants AA08459, AA06420, and AA07456.

665.3

NALTREXONE EFFECTS ON ORAL SUCROSE AND ETHANOL-REINFORCED RESPONDING IN RHESUS MONKEYS. E.D. Pakarinen[1], K.L. Williams[2] and J.H. Woods*[1,2] Departments of Pharmacology[1] and Psychology[2], University of Michigan, Ann Arbor, MI 48109-0632.

Twelve rhesus monkeys were given opportunities to respond to obtain either sucrose [3.2 - 320 mg/ml] or ethanol and water; most of the monkeys had histories of exposure to opioids (e.g., etonitazene) or phencyclidine (PCP)-like drugs. Eight of the twelve reliably self-administered ethanol relative to water over a 3-hour period. Ethanol concentration [0.25 - 32 gm/L] was altered; increases in concentration produced a bitonic preference in drug-reinforced responding relative to water and increases in ethanol intake. Individual differences in ethanol intake were considerable, and were representative of individual differences of other studies of oral ethanol intake in rhesus monkeys. Naltrexone (0.032 - 0.32 mg/kg i.m.) reduced both ethanol and water responding across monkeys, and, in some cases, reduced ethanol-reinforced responding more than water responding. At doses that reduced ethanol-reinforced drinking, naltrexone had little or no effect on sucrose-reinforced drinking. This type of study in primates may be appropriate to begin to examine parallels to the studies in humans in which naltrexone decreases certain ethanol-related behaviors. (Supported by USPHS Grants DA 00254, DA 05325, DA 07268, and DA 08568).

665.4

GENETIC DIFFERENCES IN NALOXONE ENHANCEMENT OF ETHANOL-INDUCED CONDITIONED TASTE AVERSION. J. Broadbent*, H. V. Linder, C. L. Cunningham. Medical Psychology, Oregon Health Sciences University, Portland, Oregon 97201

The influence of opioid systems on the aversive hedonic effects of ethanol was examined in alcohol-preferring (C57BL/6J) and avoiding (DBA/2J) strains of mice using the conditioned taste aversion paradigm. Naloxone (1 or 3 mg/kg) or ethanol (3 g/kg) given alone did not produce an aversion to a novel tasting fluid in C57BL/6J mice. Administration of both naloxone and ethanol, however, produced a significant taste aversion in this strain. Naloxone alone did not produce an aversion and also failed to potentiate the moderate aversion produced by ethanol (1.5 g/kg) alone in DBA/2J mice. A second experiment addressed the possibility that naloxone failed to enhance the aversion to ethanol in DBA/2J mice due to a 'floor' effect on consumption. A lower dose of ethanol (1 g/kg) produced a modest taste aversion in DBA/2J mice that, again, was not potentiated by naloxone. Additional conditioning with a high ethanol dose, however, significantly suppressed consumption indicating that naloxone's failure to enhance the effects of ethanol was not due to a 'floor' effect. These results reveal a selective enhancement of the aversive effects of ethanol in C57BL/6J mice by naloxone, suggesting that genetically determined differences in endogenous opioid systems in alcohol-preferring mice may mitigate ethanol's aversive effects.
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665.5

NALTREXONE EFFECTS ON ACQUISITION AND REDUCTION OF ETHANOL CONSUMPTION IN C57BL/6J MICE. T.J. Phillips*, C.D. Wenger, and J.D. Dorow. V.A. Med. Cntr. and Dept. of Medical Psychology, OR Health Sci. Univ., Portland, OR 97201.

Opiate receptor antagonists, such as naloxone and naltrexone, have been shown to reduce ethanol (EtOH) consumption in EtOH preferring rodent lines and strains. Most studies have concentrated on antagonist effects in animals that have already acquired high levels of EtOH drinking. We have performed three two-bottle choice studies (water vs. 10% EtOH) using the EtOH preferring C57BL/6J mouse strain. The first was designed to replicate earlier findings of naltrexone's effects on reducing established EtOH consumption. An increasing i.p. dosing regimen was used, and significant effects of naltrexone on EtOH preference and consumption were found at lower doses; these effects waned at higher doses. The second study examined the effects of chronic naltrexone on the acquisition of EtOH consumption. Time-release naltrexone pelleted mice drank more EtOH and showed greater EtOH preference than did placebo pelleted mice. The third study examined the effects of i.p. injected naltrexone on acquisition of EtOH drinking. Naltrexone injected mice consumed less and exhibited lower preferences than did saline treated mice. There appear to be important consequences of dosing parameters and possibly EtOH experience on the effects of naltrexone on EtOH consumption. Supported by grants from the Dept. of Veterans Affairs and the Alcoholic Bev. Med. Res. Found.

665.6

CENTRAL ADMINISTRATION OF AN OPIATE ANTAGONIST DECREASES ORAL ETHANOL SELF-ADMINISTRATION IN RATS. C.J. Heyser*, A.J. Roberts, G. Schulteis, P. Hyytiä, and G.F. Koob. Dept. Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Opioid peptides have been implicated in various behavioral actions of alcohol, including the reinforcing effects, however the role of specific brain sites remains to be explored. The present study examined the effects of intracerebroventricular (ICV) or intracerebral (IC) injections of an opiate antagonist on ethanol self-administration. The nucleus accumbens and central nucleus of the amygdala were selected as IC sites given that these regions have been implicated in the behavioral effects of opiate drugs. Male Wistar rats were trained in a limited access paradigm (30 min/day) to respond for ethanol (10% w/v) or water in a two-lever free-choice condition using a saccharin fading procedure. Following the establishment of stable baseline responding for ethanol (± 20%), animals were implanted stereotaxically with a guide cannula above the lateral ventricle (ICV) or with bilateral guide cannulae either above the nucleus accumbens or amygdala. Following postoperative recovery of stable baseline responding, the rats were tested 15 min after ICV or IC microinjections of an opiate antagonist methylnaloxonium (0 - 2µg). Methylnaloxonium injections dose-dependently reduced ethanol responding. Injections into the amygdala significantly reduced responding for ethanol at doses of 0.25 - 0.50 µg, whereas higher doses were required in the nucleus accumbens (0.50 - 1.0 µg) and still higher doses were needed ICV (1.0 - 2.0 µg). These results provide evidence that opioids, particularly in the amygdala, may be involved in the regulation of ethanol self-administration. Supported by grants AA08459, AA06420 and NRSA AA05403.

665.7

DECREASED VOLUNTARY ETHANOL CONSUMPTION IN TRANSGENIC MICE LACKING β-ENDORPHIN

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The opioid peptide, β-endorphin, has been purported to play a role in the voluntary consumption of ethanol. We tested this hypothesis in mice lacking β-endorphin (POMCX*4 -/- mice) due to transgenic alteration of its precursor, the proopiomelanocortin (POMC) gene. Introduction of a premature translational stop-codon into the POMC gene resulted in a truncated prohormone lacking the carboxyl-terminal region coding βendorphin, but normal expression and regulation of other peptide products of the POMC gene. We have previously demonstrated that POMCX*4-/- mice display deficient opioid stress-induced analgesia, but are not impaired in their analgesic responses to exogenously administered opiates (Rubenstein et al., submitted). In the present study, these adult male POMCX*4 -/- mice and their wild-type littermates (129/B6 hybrids) were given home-cage, two bottle choice access to either water and solutions containing either ethanol (7% vol/vol) or the selective μ-agonist, etonitazine (3mg/ml). POMCX*4 -/- mice demonstrated both a decreased preference for and consumption of ethanol compared to wild-type mice, but no differences in etonitazine self-administation were apparent. These data support the idea that β-endorphin plays a role in mediating ethanol consumption, and extend previous findings suggesting that mice lacking β-endorphin do not have altered opiate responsivity. Supported by grants from the NIH and the Markey Charitable Trust (MJL), and a VA Merit Review (JKB).

665.8

DELTA AND KAPPA OPIATE RECEPTORS MODULATE VOLITIONAL ETHANOL INTAKE IN THE RAT

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The effect of opioid antagonists on volitional ethanol intake was studied in normal Sprague-Dawley rats using a free-choice paradigm. Rats were habituated to drinking solutions with increasing concentration of ethanol, reaching a final concentration of 5% within 12 days. The rats had free access to food and water. During a four day's baseline period, the rats were deprived of water and ethanol solution for the last 4 h of the light period. The average total daily intake of ethanol during this period was 1.70±0.22 g/kg/d (n=24). An i.p. saline injection was given 30 min before the beginning of the dark period, and water and ethanol solution were reinstalled when lights went off. The average intake of ethanol during the first hour after reinstallation of water and ethanol was 0.32±0.02 g/kg/h (n=24). A four day's treatment period was initiated by i.p administration of the opioid antagonists, naltrexone (0.1 and 1 mg/kg), ICI-174,864 (0.1 mg/kg), nor-binaltorphimine (nor-BNI; 0.1 mg/kg) or saline (4 ml/kg) 30 min before water and ethanol solution were reinstalled. Naltrexone (0.1 and 1 mg/kg; unselective antagonist) reduced the ethanol intake during the one hour test sessions by 45% and 65%, respectively, compared to the baseline values. ICI-174,864 (0.1 mg/kg; δ antagonist) and nor-BNI (0.1 mg/kg: *k* antagonist) reduced ethanol intake by 47% ($p<0.01$, n=8) and 32% ($p<0.05$, n=8), respectively. Naloxonazine (0.1 mg/kg; μ_1 antagonist) had no effect on ethanol intake (n=8). The total daily intake of ethanol was unaffected by the drugs studied. Food and water intake did not differ between treatment groups and controls. Ethanol intake during the test sessions was restituted to baseline values when drugs were replaced by saline injections. The results suggest that opiate receptors of the δ and *k* subtypes modulate volitional ethanol intake in the rat, and that selective opioid antagonists may have a therapeutic potential in the treatment of alcohol dependence.

665.9

CONTENT OF PROENKEPHALIN AND PRODYNORPHIN mRNAs IN DISTINCT BRAIN REGIONS OF THE ALCOHOL-PREFERRING (AA) AND THE ALCOHOL-AVOIDING (ANA) RATS.

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A number of studies have indicated that the reinforcing effects of ethanol are mediated, at least in part, by its effects on the activity of the endogenous opioid system. Genetically determined differences in the activity of the endogenous opioid system, under basal conditions or following exposure to alcohol, may be important in controlling alcohol consumption. Indeed, previous studies have demonstrated a higher content of pro-opiomelanocortin (the precursor to beta-endorphin) mRNA in the arcuate nucleus of the AA than of the ANA lines of rats. The objective of the present studies was to investigate the presence of differences in the contents of proenkephalin and prodynorphin mRNAs in distinct regions of the brain between AA and ANA rats under basal conditions, using ^{35}S-labelled oligonucleotide probes for proenkephalin and prodynorphin and in situ hybridization techniques. Results indicated that in the arcuate nucleus and septum the content of proenkephalin mRNA was significantly higher in the AA than ANA rats. On the other hand a lower content of prodynorphin mRNA was observed in some brain regions of the AA than of the ANA rat. In conclusion, such differences in the content of mRNAs of the endogenous opioid peptides between the AA and ANA rats may be important in controlling voluntary alcohol consumption by these lines of rats.
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665.10

THE EFFECT OF AN ACUTE ETHANOL EXPOSURE ON THE RAT BRAIN POMC OPIOPEPTIDE SYSTEM.

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The discovery of endogenous opiopeptides in the central nervous system has stimulated numerous questions concerning their function. It has been suggested that they play a role in the etiology of alcoholism or other addictive diseases. Beta-endorphin 1-31 (β-end) is one of these endogenous opiopeptides. It is post-translationally processed from the inactive peptide pro-opiomelanocortin (POMC). We have previously reported that a single ethanol exposure (0.5, 1.0, and 3.0 g/kg BW, 20% w/v ethanol) resulted in an increase in β-EPLP levels in the hypothalamus and a decrease in the hindbrain, regardless of dose. Biological significance could not be attached to any individual group differences seen at several time points. In order to identify the molecular action of ethanol on this peptide system we conducted another series of experiments using male Sprague Dawley rats to study the effects of different doses of ethanol (same as before) at different time points post-ethanol administration (15, 30, 60 and 240 minutes) on changes in POMC synthesis in the hypothalamus. We measured changes in both the POMC primary transcript and the POMC mature mRNA by hybridizing hypothalamic total RNA with a POMC intron/exon junction, *in vitro* -synthesized riboprobe. No changes in primary transcript were detected. We also did not see any changes in mature POMC hypothalamic mRNA up to 4 hours post-administration at any dose. Based on the results from these two experiments we conclude that an acute ethanol exposure affects the rat hypothalamic POMC opiopeptide system by increasing levels of β-EPLP. The increase in levels appears not to be caused by an increase in POMC synthesis but rather by an increase in the post-translational processing (PTP) of the pro-peptide POMC. (Funded by DA07355)

665.11

INVOLVEMENT OF THE PEDUNCULOPONTINE NUCLEUS IN REGULATING ALCOHOL DRINKING.

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Experiments were conducted to determine if the pedunculopontine nucleus (PPN) is involved in mediating ethanol drinking behavior in the alcohol-preferring P line of rats. Female P rats were given limited access (2h/day) to 10% (v/v) ethanol and 0.0125% (g/100ml) saccharin solutions. During limited access, P rats consumed 10.4 ± 0.6 ml of ethanol and 7.3 ± 1.6 ml of saccharin. Food and water were available *ad libitum*. Cholinergic agents were microinjected unilaterally into the PPN immediately prior to ethanol access. Carbachol (1-4μg/0.5μl), which inhibits cholinergic neuronal activity within the PPN, dose-dependently decreased ethanol intake within the first 30 min (70% decrease at the highest dose; $p<0.05$). However, by the end of the 2 hr period, the actions of carbachol had begun to diminish with only the highest dose being effective. Carbachol also dose-dependently reduced saccharin intake within the first 30 minutes (90% decrease at the highest dose; $p<0.05$). The results with carbachol suggest that normal activity of the cholinergic PPN system is required for maintaining drinking behaviors in general. Scopolamine (5-15μg/0.5μl), which stimulates cholinergic neuronal activity within the PPN, dose-dependently decreased ethanol intake within the first 30 min (65% decrease at the highest dose; $p<0.05$); ethanol consumption had partially recovered by the end of the 2 hr period. On the other hand, microinfusion of scopolamine into the PPN did not alter saccharin intake after 30 minutes. The findings with scopolamine suggest that increased activity of the cholinergic PPN system selectively reduces ethanol intake, possibly by mimicking the actions of alcohol itself and consequently requiring less ethanol consumption to produce the same CNS effect. Overall, these results support an involvement of the cholinergic PPN system in regulating alcohol drinking behavior of the P line of rats. (supported in part by AA 08553, AA 07462, AA 07611).

665.12

ARE SOME OF THE EFFECTS OF ETHANOL MEDIATED THROUGH NPY?

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Central administration of NPY in low concentrations has been shown to produce anxiolysis in several anxiety models and to produce some suppression of locomotor activity at higher doses, a behavioral profile which is similar to that of ethanol. In addition, recent studies have demonstrated that ethanol can potentiate some of the behavioral effects of NPY such as NPY-stimulated food ingestion. The present study was conducted to ascertain whether NPY and ethanol have a similar electrophysiological profile and to determine if potentiation of NPY's actions can also be detected in neurophysiological measurements. Thirty-two Wistar rats were stereotaxically implanted with electrodes aimed at dorsal hippocampus (DHPC), amygdala (AMYG), and frontal cortex (CTX). Rats received the following drug regimes: saline ICV , 1 and 3 μg NPY ICV, Saline ICV plus saline IP, Saline ICV plus ethanol (0.75 g/kg) IP, NPY ICV (1 μg) plus ethanol (0.75 g/kg) IP. Forty minutes of EEG was collected and event related potentials (ERPs) recorded in response to an auditory "oddball" paradigm . Spectral analysis revealed that the higher dose of NPY produced significant ($p<.006$) overall decreases in spectral power except in the higher frequency bands (10-50 Hz) specifically in frontal cortical areas. Ethanol (0.75 g/kg) and the lower doses of NPY also decreased spectral power but not to a significant degree. The combined administration of EtOH and NPY (1μg) produced significant effects similar to those found at the 3 μg dose of NPY. Evaluation of ERPs revealed that NPY (3μg) produced reductions in the N1 component in frontal cortex ($p<.001$) and decreases in the P3 component in amygdala ($p<.05$). Low doses of ethanol were found to significantly potentiate the effects of low doses of NPY on the N1 component. These studies suggest that NPY produces electrophysiological effects similar to low doses of ethanol. In addition ethanol appears to potentiate some of the electrophysiological effects of NPY. (supported by AA 00098, 05069, and HL41910)

665.13

Dose-dependent effects of α-melanocyte-stimulating hormone (α-MSH) on the acquisition of a preference for ethanol in Sprague-Dawley rats. W. L. Nores, R. L. Bell, R. D. Olson, G. A. Olson*, and A. J. Kastin. Department of Psychology, University of New Orleans, New Orleans, LA 70148.

We studied the effects of α-MSH on the acquisition of a preference for ethanol. Thirty-six Sprague-Dawley male rats had access to a 3% v/v solution of ethanol for the first six days, followed by six days of 6% v/v solution of ethanol and six days of 12% v/v solution of ethanol in a limited access paradigm. The solutions of ethanol were available for one hour a day starting at 1600h, and intake of ethanol and water were measured at the end of this period. Food and water were freely available throughout the study, and were measured daily prior to the presentation of the solutions of ethanol. Rats were injected IP with either 0.0, 0.0001, 0.001, 0.01, 0.1, or 1.0 mg/kg α-MSH 20 minutes before the presentation of the solution of ethanol. A 6 X 3 X 6 (dose X concentration X days) mixed analysis of variance, with concentration and days as the repeated measures, yielded a significant two-way (dose X ethanol) interaction, $p < .05$. All doses of α-MSH increased intake of ethanol as the concentration of ethanol increased except the 1.0 mg/kg dose of α-MSH which decreased intake of ethanol as the concentration of ethanol increased. A significant main effect for ethanol was obtained, $p < .05$, with a higher overall intake of ethanol for the 6% and 12% v/v solutions of ethanol. There was also a significant main effect for dose, $p < .05$. An overall trend analysis on the doses of α-MSH yielded a significant quadratic trend, $p < .05$, indicating an inverted-U shaped dose-response relationship among the doses of α-MSH. The results suggest that α-MSH influences the acquisition of a preference for ethanol in rats.

665.14

TIME COURSE OF CORTICAL NMDA RECEPTOR ELEVATION AND SEIZURES IN MICE FOLLOWING WITHDRAWAL FROM CHRONIC PHENOBARBITAL. K. R. Short* and B. Tabakoff Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262, *Dept. of Psychology, Creighton Univ., Omaha, NE 68178.

The authors previously reported increased numbers of NMDA receptors in mouse cortex following chronic phenobarbital (PhB) treatment, suggesting a possible mechanism for withdrawal (W/D) seizures. We report the duration of NMDA receptor elevation following PhB W/D, and the corresponding rates of W/D seizures and levels of serum PhB.

C57BL/6 mice (N=85) ate normal chow or chow containing 2.0 g PhB/kg food (increased to 2.5 g/kg after 5 days) for 7 days, after which all mice received drug-free chow. All mice were tested for susceptibility to auditory seizures at 0, 8, 12, 24, 36, 48, and 72 hrs post-W/D. PhB-treated mice were sacrificed at either 0, 12, 24, 36, or 72 hrs post-W/D, and control mice at 24 hrs and 72 hrs. At sacrifice, trunk blood was assayed for serum PhB and cortical tissue was prepared for NMDA receptor quantification. [^{3}H]MK-801 binding (0.25 to 25 nM) to crude synaptic membranes was determined in the presence of 10 mM glutamate, 1 mM glycine, and 100 μM $MgSO_4$ in the presence or absence of 1 mM MK-801.

No PhB-treated mice displayed seizures at 0 or 8 hrs post W/D, while 67% at 12 hrs and 64% at 24 hrs showed W/D seizures, after which time seizure rates dropped sharply. Serum PhB at 0 hrs was 102μg/ml but dropped to 3 μg/ml at 12 hrs W/D. B_{max} for MK-801 binding was 49% higher than controls at 0 hrs W/D (K_d unchanged) and was still elevated 24% at 24 hrs, but dropped to control levels at 36 hrs post-W/D. These results suggest PhB W/D seizures are associated with increased NMDA receptor number and low serum PhB. (AA-09014, AA-09005, AA-07464)

665.15

NITRIC OXIDE SYNTHASE INHIBITION REDUCES ALCOHOL WITHDRAWAL HYPERMOTILITY, ALCOHOL PREFERENCE AND CORTICAL HYPERVASCULARIZATION. Ph. De Witte*, C. Verheyden and F. Lallemand. Lab. Psychobiology, Univ. of Louvain, Belgium.

Nitric oxide (NO) modulates the vascular system and also interacts with alcohol. L-NNA, a stereoselective NO synthase inhibitor, was mixed with water in the drinking bottle at 5mg/kg/day during pulmonary chronic alcoholization. Rats were kept for 30 days in the alcoholization chamber before recording of 1)the motility during the withdrawal syndrome 2) the preference for ethanol in a free choice water versus 10% (v/v) ethanol solutions and finally 3) the microvascular morphometric quantification of the vessels length in the fronto-parietal cortex. Results showed that 1) the hypermotility observed during the withdrawal syndrome was significantly decreased in L-NNA rats 2) the alcohol preference cut-off, i.e. the moment when rats failed to drink preferentially alcohol, was significantly decreased in the L-NNA treated rats, while no difference occured in the global liquid consumption between treated and untreated rats and 3) the hypervascularization of the cortical area observed after chronic alcoholization was significantly decreased in L-NNA treated rats. L-NNA, a NO synthase inhibitor, reduced thus the behavioral dependence after alcoholization, the behavioral preference for alcohol as well as the microvasculature length of the cortex supporting thus the direct implication of NO in alcohol abuse and its withdrawal.

665.16

INDOMETHACIN INCREASES ALLOPREGNANOLONE PLASMA LEVELS AND REVERTS ETHANOL PSYCHOPATHOLOGY IN ALCOHOLICS WITHDRAWAL. E.Romeo*, F.Pompili°, F. di Michele°, G.Spalletta°, P.Fucci°, C.Fumari°, M.Pace°°, L.Masala°°, G.Vaccarella°°and A. Pasini.°, Dept. Med. Sperimentale and °Dept. San. Pubblica, "Tor Vergata" Univ., Rome; °°"S. Giuseppe" Hosp. ,Albano, Rome , Italy 00133

We previously reported (Neuroscience 1994, Abst.215.26) a large decrease of plasma allopregnanolone (ALLO) levels in alcoholics in the early phase of ethanol withdrawal that negatively correlated with symptoms of anxiety and depression. Here we treated a group of alcoholics during withdrawal, with indomethacin (100mg/day) that inhibits a 3α-hydroxysteroid-oxidoreductase (HSOR), responsible for the conversion of ALLO to 5α-dihydroprogesterone (DHP). The plasma levels of progesterone (PROG), ALLO and DHP were measured by gas chromatography-mass spectrometry. Blood samples were taken on the first day of withdrawal (just before the beginning of treatment with indomethacin), each of the next 4 days of treatement and on day 15th (treatment free). On the same days patients were administered psychometric tests to measure depression, anxiety and agressiveness. On the first day of indomethacin treatment plasma ALLO increased 10 fold compared to control subjects. On the following 3 days and on day 15th ALLO levels were as in control subjects but higher than in alcoholics untreated. Since the plasma levels of PROG were unchanged the data support the hypothesis that indomethacin reverts the decrease of ALLO plasma content observed in not treated patients by acting on the HSOR. Although all subjects met the DSM-IV criteria for alcohol dependence, measurements of anxiety and depression in the indomethacin treated patients were within normal values. These findings raise the possibility that pharmacological interventions aimed to increase ALLO content could be beneficial in the treatment of the withdrawal syndrome in alcoholic patients.

665.17

RELATIONSHIP BETWEEN SPONTANEOUS SEIZURE OCCURRENCE AND SALICYLATE SPIN-TRAP ADDUCTS FROM WHOLE RAT BRAIN HOMOGENATES AFTER SINGLE AND REPEATED ETOH DEPENDENCE EPISODES. M. Vallett[1],* D.V. Gauvin[1], W.W. Beatty[1], T. Tabatabaie[2] & R.A. Floyd[2], Dept. Psychiatry & Behavioral Sciences, University of Oklahoma Health Sciences Ctr., [1], Dept. of Free Radical Biology & Aging, Oklahoma Medical Research Foundation[2], Oklahoma City, OK 73190

ETOH exposure increases free-radical generation, lipid peroxidation (LP), and [GSSG] with concomitant decreases in [SOD] and [GSH] in rat brain. The increase in LP is independent of acetaldehyde production by both alcohol dehydrogenase and catalase (Montoliu *et al.*, 1994, *J Neurochem*, 63, 1855-1862). The present study examined the relationship between the occurrence of ETOH-withdrawal-associated spontaneous convulsive behaviors and the level of CNS hydroxyl-radical species in rats exposed to either single or multiple (11) cycles of ethanol vapor exposure-induced dependency. HPLC assays of salicylate hydroxylation products (catechol, 2,3 Dihydroxybenzoate [2,3 DHBA], and 2,3 Dihydroxybenzoate [2,5 DHBA]) were used to quantify levels of brain homogenate hydroxyl-radical concentrations 12 hours after removal from the ETOH chambers. Water control and ethanol treated rats (terminal BAC's of ≥ 300 mg/dl for >24 hrs) were injected with 100 mg/kg salicylate, as a trapping agent, one hour before rapid decapitation, tissue harvesting, and liquid nitrogen tissue immersion. Significant group differences ($F[1,22]=22.7$, $p<.001$) were found in 2,3 DHBA concentrations after a single exposure cycle. Significant group-dependent differences were also found for both 2,5 DHBA concentrations and the total DHBA:salicylate ratio after both single and multiple exposures. Point biserial correlations between the occurrence of spontaneous withdrawal seizures and the concentration levels of 2,5 DHBA and the total DHBA:salicylate ratios were 0.82 ($t_{calc}(15)=7.4$, $p<.001$) and 0.75 ($t_{calc}(15)=5.9$, $p<.001$), respectively.

665.18

CALCIUM CHANNEL BLOCKERS REDUCE ETHANOL WITHDRAWAL-INDUCED ANXIETY IN MALE RATS. C.J. Wallis*, and H. Lal. Department of Pharmacology and SAINT, University of North Texas Health Science Center, Fort Worth, TX 76107.

Ethanol modifies the activity of a variety of ligand gated and voltage regulated ion channels. If increased calcium channel activity contributes to the ethanol withdrawal (EW) syndrome, then reducing calcium channel activity during EW should ameliorate some of the symptoms of ethanol withdrawal. In the present studies, we investigated the efficacy of nitrendipine and nimodipine, calcium channel blockers, to modify an EW symptom in an animal model of anxiety: male rat behavior in elevated plus-maze (EPM). Long-Evans hooded male rats were given a nutritionally balanced liquid diet containing 4.5% ETOH for 10 d (Lal et al., JPET 247:508, 1988). Twelve h after removal of the ETOH diet, rats were tested. We observed a significant reduction in the open-arm activity and the number of total arm entries indicative of EW. Single pretreatment (1h) with nitrendipine or nimodipine (0.08-5.0 mg/kg) resulted in a dose related increase in open arm activity at doses of 1.25-5.0 mg/kg. Acute pretreatment did not improve the total number of arm entries. Repeated injections of nitrendipine or nimodipine (1.25-5 mg/kg, 2/day, 3 or 5 days) increased open arm activity and total arm entries during EW. These data support a role for increased Ca^{2+} channel activity in EW. Supported by NIAAA grants AA06890 and AA09567

665.19

EFFECTS OF CHLORDIAZEPOXIDE AND ACAMPROSATE ON THE CONDITIONED PLACE AVERSION INDUCED BY ETHANOL WITHDRAWAL. B.A.Baldo*, C.J. Heyser, P.Griffin, G. Schulteis, L. Stinus#, and G.F. Koob. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA. 92037 and #Universite de Bordeaux II, Bordeaux, France..

Ethanol withdrawal is associated with irritability, anhedonia, anxiety, and other affective symptoms. In rats, ethanol withdrawal has been found to produce elevated brain stimulation-reward thresholds, locomotor hyperactivity, hypothermia, reduced seizure threshold, and conditioned place aversion. In the present study, an attempt was made to reverse ethanol withdrawal-induced conditioned place aversion by the classic benzodiazepine anxiolytic chlordiazepoxide (5 mg/kg, 10 mg/kg), and the novel anti-craving drug, acamprosate (400 mg/kg). Male Wistar rats were maintained on an ethanol-containing liquid diet (10% ethanol). After 20-24 days of exposure to the ethanol liquid diet, animals were tested in the place conditioning apparatus, which consisted of three distinct rectangular compartments separated by a triagular central region. During this initial exposure, animals freely explored all three compartments of the apparatus. One to three days later, animals were confined to one of the compartments while not undergoing withdrawal. On the following day, liquid diet was removed and the rats were exposed to a second compartment 8 hrs into withdrawal (mean BAL at withdrawal: 218 mg%), following injection with vehicle, chlordiazepoxide, or acamprosate. When subsequently allowed to re-explore all compartments, animals showed a significant place aversion to the compartment associated with ethanol withdrawal; both doses of chlordiazepoxide reversed this effect. In an initial attempt to characterize the effect of acamprosate on ethanol withdrawal-induced place aversion,five out of eight vehicle-treated rats displayed a reduction in the withdrawal-paired compartment, whereas none of the acamprosate-treated animals demonstrated such an effect. Thus, both chlordiazepoxide and acamprosate appear to reverse the place aversion elicited by ethanol withdrawal.

665.20

REPEATED EPISODES OF ETHANOL WITHDRAWAL LOWER PENTYLENETETRAZOL (PTZ) SEIZURE THRESHOLD DOSE IN MICE. J.L. Diaz-Granados*, R.R. Reich, K.G. Fernandes, and H.C. Becker. Med. Univ. of S. Carolina and VAMC, Charleston, S.C. 29425.

We have established a model in which multiple episodes of EtOH withdrawal result in an exacerbation of handling-induced convulsions (HIC) in adult C3H mice compared to mice that have been withdrawn a single time. The present experiment was designed to examine whether repeated EtOH withdrawals alters sensitivity to the proconvulsant effects of the GABAa receptor antagonist PTZ, as assessed by the tail vein infusion method. Mice were divided into three treatment groups: a multiple withdrawal (MW) group received 4 cycles of 16 hr EtOH vapor separated by 8 hr periods of abstinence; a single withdrawal (SW) group received a single 16 hr bout of EtOH; and controls (C) received no EtOH. At withdrawal, blood ethanol levels did not differ among the ethanol-exposed groups (150-175 mg/dl). Separate groups of animals were administered PTZ (4 mg/ml) i.v. either 8 or 24 hr post-withdrawal. At the 8 hr timepoint, threshold doses for tonic/clonic (TC) and tonic hindlimb extension (THE) were significantly lower in the MW group as compared to the SW and C groups (TC: 17.9 ± 1.3, 40.5 ± 7.7, 38.6 ± 7.2 mg/kg, respectively; THE: 20.5 ± 1.3, 50.9 ± 10.8, 80.7 ± 11.8 mg/kg, respectively). In addition, these treatment groups differed in the development of the seizure where the time between the myoclonic jerk (MJ) and TC was significantly less in the MW as compared to SW and C groups (6.2 ± 1.1, 35.9 ± 10.1, 33.0 ± 9.6 sec, respectively). At the 24 hr timepoint, preliminary results indicate a lower seizure threshold dose to MJ in the MW group. The latter endpoints are currently being evaluated. These results suggest that modifications at the GABAa receptor complex may underlie the potentiated seizure activity resulting from repeated ethanol withdrawals. Supported by NIAAA and VAMC.

665.21

ß-CARBOLINES DURING DETOXICATION AND ABSTINENCE IN CHRONIC ALCOHOLICS - A LONGITUDINAL STUDY N. Wodarz*, H. Rommelspacher[1], G.A. Wiesbeck, P. Riederer, J. Böning Department of Psychiatry, University of Würzburg, Füchsleinstr. 15, 97080 Würzburg, Germany; [1]Dept. of Neuropsychopharmacology, Free University of Berlin, Ulmenallee 30, 14050 Berlin, Germany

ß-carbolines (BC) could be formed by condensation of indolealkylamine and catecholamine transmitters with aldehydes or pyruvate. It was hypothesised that BCs might contribute to the pathogenesis and manifestation of at least some phenomena of addictive alcohol drinking. Chronic intraventricular infusion of BCs have repeatedly been shown to increase voluntary alcohol ingestion in rats and monkeys. Moreover, ethanol-preferring rats exhibit an increased formation, increased levels in brain, and increased excretion of BCs. However, few studies investigating the role of neurotransmitter condensation products in alcoholism of man have been conducted. Therefore, we investigated 12 men (age 23 - 58 years), fulfilling at least 6 of 8 diagnostic ICD-10 criteria of alcohol dependence, lasting for at least 5 years. Relevant psychiatric and/or somatic comorbidity was excluded. Levels of BCs Harmane and Norharmane were measured repeatedly in plasma and 24h-urine. Moreover, possible correlations of BC kinetics to life events, incl. history of alcoholism, personality traits, and actual psychopathology were evaluated. Compared to 12 age-matched healthy men without any history of dependence Harmane- and Norharmane-levels in 24h-urine were significantly increased. Harmane decreased during abstention without reaching values of healthy subjects. Norharmane exhibited an additional increase after alcohol withdrawal and remained elevated even 8 weeks after detoxication. Preliminary results indicate correlations of BC-levels with some demographic, clinical, and neuropsychological data.

DRUGS OF ABUSE: ALCOHOL V

666.1

ALCOHOL-ILLNESS ASSOCIATIONS IN THE SELECTIVELY BRED AA AND ANA RATS. Richard L. Elder*, Illinois State University, Campus Box 4620, Normal, IL 61790-4620 and Nancy Badia-Elder, and Stephen W. Kiefer. Department of Psychology, Kansas State University, Manhattan, KS 66506-5302.

Selectively bred AA and ANA rats were used to examine the acquisition and extinction of an alcohol-illness association. Each animal was given one pairing of a 6% (v/v) alcohol solution followed by intubation of a 0.15 M LiCl solution. On the day following the acquisition trial, animals were tested for taste reactivity to 6% alcohol. The animals then received seven extinction trials with the alcohol solution, each trial given every other day. Analysis of the taste reactivity data showed that AA rats produced significantly more ingestive responses and fewer aversive responses than ANA rats. Although both AA and ANA rats showed alcohol avoidance on the initial extinction test, the AA rats consumed significantly more alcohol over the course of the seven extinction trials. These results suggest that, while AA rats acquired an avoidance of alcohol when it was paired with illness, the avoidance did not appear to entail a shift in palatability nor was it as durable as that found in ANA rats.

666.2

TASTE REACTIVITY TO ALCOHOL IN RATS SELECTIVELY BRED FOR SENSITIVITY TO A SEROTONIN-1A AGONIST. N.E. Badia-Elder*, S.W. Kiefer, D.H. Overstreet, and A.H. Rezvani. Kansas State University, Manhattan, KS 66506-5302, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7178.

Rats selectively bred for high (HI) and low (LO) hypothermic responses to the serotonin-1A agonist 8-hydroxy-(2-amino)-tetralin (8-OH-DPAT) were tested for taste reactivity to five concentrations of alcohol (5%, 10%, 20%, 30%, and 40%), sucrose, quinine, and water both prior to and following 21 days of two-bottle consumption testing with 10% alcohol and water. Both groups showed similar increases in ingestive and decreases in aversive responding to alcohol from the first to second set of taste reactivity tests. However, HI and LO rats did not differ in taste reactivity responding in either pre- or post-consumption tests. During consumption testing, HI rats consumed only slightly more alcohol than LO rats. The results indicated that HI and LO rats do not differ greatly in alcohol reactivity or consumption. However, after experience with alcohol, both rat lines showed a hedonic shift in alcohol palatability as shown by taste reactivity tests. Because these lines differ greatly in hypothermic responses to 8-OH-DPAT it seems unlikely that serotonin-1A receptors play a major role in modulating alcohol reactivity or consumption. (Supported by Training Grant T 32 MH 19547 from NIMH to the Society for Neuroscience).

666.3

ALCOHOL RESPONSES IN NULL MUTANT MICE MISSING THE SEROTONIN $5HT_{1B}$ RECEPTOR. J.C. Crabbe*, T.J. Phillips, D.J. Feller, N. Castanon and R. Hen. VA Med. Ctr. and Oregon Hlth. Sci. Univ., Portland, OR 97201 and Columbia Univ. P&S, NY, NY 10032.

Quantitative Trait Loci analyses suggest that the $5HT_{1B}$ serotonin receptor subtype may be a candidate gene that influences several responses to ethanol (EtOH). Mutant mice have been developed that lack the gene coding for the $5HT_{1B}$ receptor. Pharmacological evidence suggests that these mice might show reduced sensitivity to several EtOH effects. Homozygous knockouts ($5HT_{1B}^{-/-}$) were compared with heterozygotes ($5HT_{1B}^{+/-}$) and wild-type ($5HT_{1B}^{+/+}$) controls for EtOH-induced hypothermia and tolerance. (-/-) and (+/-) mice had diminished response to an acute 3 g/kg EtOH injection as compared with (+/+) mice. With 3 daily injections, tolerance developed in all genotypes to an equivalent extent. Because the hypothermia data suggested partial loss of response in heterozygotes even though they are thought to have normal $5HT_{1B}$ receptor levels, we tested additional animals after 10 mg/kg RU 24969, a $5HT_{1B}$ receptor agonist. (+/+) animals had a large, and (-/-) mice a very small hypothermic response: heterozygotes had an intermediate response. One week later, these mice were tested for their acute withdrawal response following a single 4 g/kg dose of EtOH, but the three genotypes did not differ. Other effects of EtOH were also tested. These studies are consistent with a role for serotonergic systems in modulating responsiveness to some, but not all, effects of EtOH. Support by NIAAA, NIDA, & Dept. of Veterans Affairs.

666.4

POTENTIATION OF ETHANOL-INDUCED EXCITATION OF VENTRAL TEGMENTAL AREA NEURONS BY SEROTONIN IS MEDIATED BY $5\text{-}HT_2$ RECEPTORS. M.S. Brodie* and R.D. Trifunović, Dept. of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL 60612-7342.

The dopamine-containing neurons of the ventral tegmental area (VTA) may be important in mediating the rewarding properties of drugs of abuse. We have previously shown that serotonin enhances ethanol-induced excitation of putative dopaminergic neurons of the VTA. Serotonin receptors have been classified into a number of different subtypes, therefore, we further characterized serotonin potentiation of ethanol-induced excitation with selective agonists and antagonists. Brain slices containing the VTA were prepared from male Fischer 344 rats, and all drugs were administered in the superfusate. Ethanol (40 - 160 mM) produced a concentration-dependent increase in the spontaneous firing rate, and serotonin (5 - 10 μM) enhanced this excitation. Two serotonin $5\text{-}HT_2$ agonists, DOI (0.1 - 1 μM) and α-methylserotonin (0.5 - 5 μM) also enhanced ethanol-induced excitation. The enhancement of ethanol-induced excitation was completely reversed by the administration of ketanserin (0.5 - 2 μM), a selective serotonin $5\text{-}HT_2$ antagonist. In some experiments, ethanol-induced excitation was reduced by ketanserin, suggesting that endogenous serotonin may contribute to the magnitude of ethanol-induced excitation seen in this preparation. These experiments indicate that potentiation of ethanol-induced excitation of VTA neurons is produced by serotonin acting at $5\text{-}HT_2$ receptors. Support: PHS grant AA-09125-03.

666.5

ETHANOL- AND NICOTINE-PREFERRING TRANSGENIC MICE DISPLAY REDUCED SEROTONIN CONTENT IN NUCLEUS ACCUMBENS. R.W. Steger*, C.J. Meliska, C.T. Fadden and A. Bartke. Dept. of Physiology, SIU School of Medicine, Carbondale, IL 62901.

Male transgenic (T) mice overexpressing the bovine growth hormone gene consume more and exhibit greater preferences for ethanol (EtOH) and nicotine solutions than non-transgenic litter mate controls (Pharmacol. Biochem. Behav. 50:563,1995). Since mesolimbic forebrain monoamines may modulate drug-induced reinforcement, we examined indices of content and turnover of serotonin (5-HT) and dopamine (DA) in various brain regions in male T and non-T mice, one hour after i.p. injection of either saline or 1.5 g/kg EtOH. Results showed that 5-HT content in nucleus accumbens (Nacc), but not corpus striatum was lower in T than in non-T mice (1.7 ± 0.5 vs. 4.6 ± 1.0 ng/mg tissue, $P < 0.01$), one hour after i.p. saline. In contrast, one hour after i.p. EtOH, T and non-T mice did not differ in Nacc 5-HT content (4.4 ± 1.3 vs. 4.7 ± 1.4 ng/mg tissue, $P > 0.05$), suggesting that EtOH normalized 5-HT levels in T mice, while having no effect in non-T controls. Levels of 5-hydroxyindole acetic acid after saline and EtOH showed a similar pattern of results. T and non-T mice did not differ in DA content in Nacc or corpus striatum after saline or EtOH. Reduced 5-HT function in reward-relevant brain regions may be associated with increased vulnerability to drug-induced reinforcement. (Supported by NIAA Grant 1-R03-AA09457.)

666.6

DIFFERENTIAL IMPACT OF ETHANOL ON EXTRACELLULAR LEVELS OF SEROTONIN AND GLUTAMATE: A COMPARATIVE STUDY IN LEWIS AND FISCHER 344 RAT STRAINS. M. Selim* and C. W. Bradberry. Yale Univ. Sch. of Med., Depts. of Psychiatry and Laboratory Medicine, and the West Haven Veterans Administration Hospital, Box 116/A2,West Haven, CT 06516.

The present study investigated the impact of systemic ethanol administration on extracellular levels of 5-HT and Glutamate (Glu) in the prefrontal cortex (PFC) and the nucleus accumbens (NA) in Lewis (LEW) and Fischer (F/344) inbred rat strains using microdialysis in awake animals. Lewis rats self-administer alcohol at higher rates than F/344 rats and are thought to be genetically predisposed to drug abuse. The extracellular levels of 5-HT and Glu in the PFC of either strain were not significantly altered by i.p. ethanol at 0.5, 1.0 or 2.0 g/kg; $p>0.05$ in all cases. Similarly, injections of ethanol at 0.5 or 2.0 g/kg did not result in any significant change ($p>0.05$) in NA dialysate concentrations of 5-HT or GLU in either strain. At 1 g/kg, ethanol caused a 44%±16 increase in NA-dialysate 5-HT levels in LEW rats ($p=0.006$). Again, no significant change was observed in F/344 rats ($p=0.66$). Preliminary data indicate that ethanol (at 1g/kg) causes a non-significant trend toward an increase in extracellular Glu in NA in both strains. Basal levels of Glu in both the NA and PFC of LEW rats were significantly lower than in F/344 rats ($p=0.05$ and 0.04, respectively). Basal levels of 5-HT were also lower in LEW rats, but the levels of statistical significance were marginal ($p=0.06$ and 0.08). These findings indicate that responsiveness of 5-HT to ethanol, or basal levels of Glu may be important in strain differences between F344 and Lewis rats in response to acute ethanol administration. Supported by DA 08073, DA 08227, The Yale VA Alcoholism Research Center, and the West Haven VA Center for the study of PTSD.

666.7

ETHANOL EFFECTS ON EXTRACELLULAR DOPAMINE IN NUCLEUS ACCUMBENS: COMPARISON BETWEEN FISCHER AND LEWIS RAT STRAINS. Z. Mocsary and C.W. Bradberry*, Yale Univ. Sch. of Med., Depts. of Psychiatry and Laboratory Medicine, and the West Haven Veterans Administration Hospital, West Haven, CT 06516.

Increasing attention has focused upon comparisons between strains of animals with differing propensities to consume alcohol and other drugs of abuse in order to gain insight into potential biochemical bases for drug abuse vulnerabilities. This includes the Fischer and Lewis rat strains, with the Lewis rats exhibiting greater ease of self-administration of ethanol, cocaine and opiates. The neurochemical basis of ethanol reward is unclear, without the same dependence upon intact dopamine (DA) innervation of the nucleus accumbens as is seen with psychostimulants. We have made a microdialysis comparison of the effects of ethanol upon both extracellular DA, and serotonin (accompanying poster) in awake animals of the Fischer and Lewis rat strains. In the nucleus accumbens, there was no significant increase in DA in either strain at 0.5 g/kg (i.p.). A 1.0 g/kg dose caused a significant increase only in the Fischer strain (180 % of baseline at 60 min post-ethanol), with a significant difference in response between the two strains (by two-way repeated measures ANOVA). The 2.0 g/kg dose caused a nearly significant increase in the Fischer strain, no significant increase in the Lewis, and no significant difference in responsiveness between the two strains. Basal extracellular DA levels were not different between the two strains. Our results are not consistent with an elevated mesoaccumbens DA response to ethanol as a basis for the enhanced preference of Lewis rats for ethanol. Supported by DA 08073, DA 08227, DA 04060, The Yale VA Alcoholism Research Center, and a NARSAD Young Investigator Award to CWB.

666.8

ALCOHOL SELF-ADMINISTRATION AND AGGRESSION IN RATS: DOPAMINE AND SEROTONIN IN N. ACCUMBENS. A.M.M. van Erp* and K.A. Miczek. Department of Psychology, Tufts University, Medford, MA 02155

Ethanol, when self-administered orally, enhances aggression in a subpopulation of resident rats confronting an intruder. These residents were characterized neurochemically, using *in vivo* microdialysis of dopamine and serotonin in the n. accumbens. Male Long-Evans rats, housed with a female, attacked a smaller male intruder in their home cage during 5 min tests. Rats which consistently attacked were trained to drink a 10% ethanol solution during 15 min access in their home cage, using a sucrose-fading technique. After ethanol intake stabilized, intruder tests were conducted 2/wk, starting 5 min after the ethanol session. Blood samples were taken directly after the fight from the orbital sinus, under isoflurane anesthesia. Under these conditions, rats drank up to 1.0 g/kg, resulting in BAC levels in a range of 10-80 mg/dl, but mostly 20-40 mg/dl. Aggressive behavior after ethanol self-administration was enhanced in some animals (n=5) and unchanged in others (n=3), similar to previously reported results from tests with experimenter-administered ethanol. In a second experiment changes in catecholamines in the nucleus accumbens were monitored during ethanol drinking and subsequent intruder tests. Ten min samples were taken during 60 min baseline, 10 min ethanol exposure, 10 min intruder test and 120 min recovery. During the intruder test the residents displayed circa 15 bites and threats, for a total duration of 140 seconds. Dopamine release was increased during ethanol self-administration and the subsequent intruder confrontation; no change or even a decrease in dopamine is observed during and after intruder confrontations without ethanol. Preliminary results suggest that serotonin levels follow a similar pattern. Individual differences in neurochemical measurements are evaluated in relation to ethanol's aggression enhancing effects.

666.9

COMPARISON OF THE EFFECTS OF INTRAPERITONEAL, INTRAGASTRIC, AND DIRECTLY PERFUSED ETHANOL ON EXTRACELLULAR DOPAMINE LEVELS IN NUCLEUS ACCUMBENS OF LEWIS RATS. J. Chen*, J. Li and E.L. Gardner, Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, New York, NY 10461

Enhancement of synaptic activity in the nucleus accumbens (Acc) terminal projections of the mesolimbic dopamine (DA) system is one of the few neuropharmacological commonalities among drugs of abuse (Chen, *Sem Neurosci* 5:315-320, 1993), and drug action on this system is hypothesized to be an essential neurobiological substrate for addiction (Wise, *Pharmacol Biochem Behav* 13[suppl.1]:213-223, 1980; Wise & Bozarth, *Brain Res Bull* 12:203-208, 1984). However, ethanol's actions on these brain systems are less clear. Even the effect of different routes of ethanol administration on extracellular Acc DA levels remains unclear, as does interaction between route of administration and self-administration versus exogenous administration. We studied the effect of three routes of ethanol administration on behavior and Acc DA overflow (by *in vivo* brain microdialysis) in ethanol-preferring Lewis rats. Intraperitoneal (i.p.) ethanol injection (0.5, 1.0 g/kg) and intragastric ethanol gavage (0.5, 1.0, 2.5, 10.0 g/kg) produced significant motor effects (ataxia, etc.), but direct Acc ethanol perfusion (0.01, 0.1, 1.0, 5.0% v/v) did not. Acc ethanol perfusion and i.p. ethanol injection produced significant increases in Acc DA overflow, but intragastric ethanol gavage did not. Intragastric ethanol consumption may require volitional self-administration to activate Acc reward synapses (Moolten & Kornetsky, *Alcohol* 7:221-225, 1990). (Supported by NIAAA grant AA 09547, NIDA grant DA 03622, and the Aaron Diamond Foundation).

666.10

THE EFFECT OF REPEATED ADMINISTRATION OF ETHANOL ON THE RELEASE OF DOPAMINE IN THE NUCLEUS ACCUMBENS OF THE ALCOHOL-PREFERRING AA AND ALCOHOL-AVOIDING ANA RATS. K. Kiianmaa*, M. Nurmi[1], T. Ashizawa and J.D. Sinclair. Biomedical Research Center, Alko Group Ltd, and [1]Department of Biosciences, University of Helsinki, Helsinki, Finland.

The aim of the study was to investigate the effect of repeated administration of ethanol on the release of dopamine in the nucleus accumbens of the alcohol-preferring AA (Alko Alcohol) and alcohol-avoiding ANA (Alko Nonalcohol) rats with *in vivo* microdialysis. One group of AA rats self-administering ethanol (group AA-SA) was offered a two bottle choice between ethanol (10% v/v) and water. A yoked group of AA rats (group AA-IG) and another of ANA rats (group ANA-IG) received intragastrigally at 8:00, 13:00 and 17:00 daily the amount of ethanol (g/kg) drunk by their matched pair. After two weeks of continual access, the AA-SA group was switched to daily 30 min limited access to ethanol at 13:00, while the yoked groups were intubated once daily. After three weeks of limited access to ethanol the rats were implanted with a guide cannula for *in vivo* microdialysis, and the effect of a challenge dose (1 g/kg, IP) of ethanol on the release of dopamine in the nucleus accumbens was studied. Samples were collected from freely moving animals every 10 minutes, and concentrations of the monoamines and their metabolites were determined in the dialysate with small bore HPLC. Ethanol increased the release of dopamine in a similar manner in the AA-IG and the ANA-IG groups, while the effect of ethanol on the release of dopamine in the AA-SA group was significantly smaller than in the AA-IG group. The results show that there was no difference between the AA and ANA rat lines in the effect of prior intragastric ethanol treatment on the subsequent release of dopamine by ethanol. In contrast, prior self-administration of ethanol decreased the response to ethanol suggesting that the manner of exposure to ethanol can alter the neurochemical reaction.

666.11

TIME-DEPENDENT EFFECTS OF ACUTE ETHANOL ADMINISTRATION ON REGIONAL CEREBRAL BLOOD FLOW IN THE RAT. D. Lyons*, M.D. Miller, A.M. Crane, A.A. Hedgecock, S.L. Hart & L.J. Porrino. Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Medical Center Blvd., Winston-Salem, NC 27157.

The present study tested the hypothesis that the effects of ethanol administration on brain activity depend upon the length of time following administration. A number of reports, for example, support the assertion that the rewarding effects of alcohol intake coincide with the ascending limb of the blood alcohol curve (BAC) and the depressant effects coincide with the descending limb. To understand better the neurobiology of ethanol intake, we evaluated the functional consequences of acute administration in awake rats at 5 and 15 minutes after treatment (1.0 g/kg ethanol or saline, ip), using the quantitative [^{14}C]iodoantipyrine method for measurement of regional cerebral blood flow. These time-points were chosen because, although the 5 min point is on the ascending limb and the 15 min point is on the descending limb of the BAC, the same blood ethanol levels exist at each point, thus reducing potential differences related to the bioavailability of ethanol. Findings indicate that blood flow increased at 5 min compared to controls in the olfactory tubercle, basolateral amygdala and motor cortex, and these values returned to baseline at the 15 min time-point. Blood flow was altered at 15 min, however, in the caudate, nucleus accumbens and hippocampus. Other brain regions were unaffected by treatment. These data are consistent with work from this laboratory measuring the effects of ethanol on cerebral metabolism and demonstrate that the pattern of brain activity is different on the ascending and descending limbs of the BAC despite similar blood levels of ethanol. *(Supported by NIAAA grant AA09291)*

666.12

A STRATEGY FOR ASSESSMENT OF THE CONTRIBUTION OF DOPAMINE TO THE CHANGES IN CEREBRAL METABOLISM FOLLOWING ETHANOL ADMINISTRATION L. Williams-Hemby* and L.J. Porrino. Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

The administration of methylphenidate (3.0 mg/kg/IV), an indirect dopaminergic agonist, altered rates of local cerebral glucose metabolism as measured by the 2-[^{14}C] deoxyglucose (2DG) method in structures of the mesocorticolimbic and nigrostriatal dopamine systems including the nucleus accumbens, olfactory tubercle, globus pallidus, entopeduncular nucleus, and subthalamic nucleus. Although these changes occur in regions that receive dopaminergic innervation, they cannot be directly attributed to increased dopaminergic activity because the 2-DG method is not specific to a given neurotransmitter system. In order to determine whether the increases in glucose metabolism in these structures are due to increases in dopaminergic activity, the non-specific dopamine antagonist flupenthixol was administered in conjunction with methylphenidate to block dopaminergic activity. The administration of flupenthixol (0.1 mg/kg/IP) 2.5 hours prior to the administration of methylphenidate blocked these regional alterations in cerebral metabolism. These data demonstrate the feasibility of this pharmacological approach for the identification of neurotransmitter-specific changes in functional activity. This strategy is also being used to examine the neurochemical nature of the increases in glucose metabolism in the mesocorticolimbic dopamine system observed following the oral administration of ethanol. Supported by grants AA09346 (LJP) and AA05400-01 (LWH).

666.13

DOPAMINERGIC DYSFUNCTION AS RESIDUAL MARKER OF ALCOHOLISM. A. Heinz*, S. Kuhn, T. Sander, H. Harms, L.G. Schmidt, H. Rommelspacher. Dept. of Psychiatry, Free University of Berlin, 14050, Germany.

Alcoholics with poor treatment outcome display reduced sensitivity of dopamine receptors before detoxification, compared to patients with good treatment outcome and healthy controls (Heinz et al., Alc. Clin. Exp. Res. 19, 62-65, 1995). Therefore, we tested the hypothesis that the sensitivity of central dopamine receptors (apomorphine-induced Growth Hormone (GH) release) corresponds to the allelic constitution of DNA-polymorphisms of the dopamine D1 (D1.7) and D2 (TaqA) receptor genes and allows to predict treatment outcome. Forty-seven alcohol-dependent patients were observed for six months after detoxification. Patients with poor treatment outcome displayed blunted GH response compared to subsequent abstainers. In these patients, a strong correlation between dopamine receptor sensitivity and the amount of chronic ethanol consumption was found. On the other hand, GH release and treatment outcome did not differ in patients carrying different alleles of the examined dopamine D1 and D2 receptor gene polymorphisms. We conclude that dopamine receptor dysfunction in alcoholics is a residual, not a trait marker of alcoholism.

Supported by the Deutsche Forschungsgemeinschaft (Az He 916/7-2)

666.14

INVOLVEMENT OF D_2 RECEPTORS IN THE SUPPRESSIVE EFFECT OF THE TRH ANALOG TA-0910 ON ALCOHOL INTAKE. Amir H. Rezvani*, J.C. Garbutt and G.A. Mason. Skipper Bowles Center for Alcohol Studies and Dept. of Psychiatry, UNC Sch. of Med., Chapel Hill, NC 27599.

Previously, we showed that the thyrotropin-releasing hormone analog TA-0910 dose-dependently reduces alcohol intake in alcohol preferring (P) rats. Further, we observed cross-tolerance between TA-0910 and bromocriptine in reducing alcohol intake, suggesting the involvement of dopaminergic systems. In the present study, experiments were conducted to determine the neuronal mechanisms underlying the attenuating effect of TA-0910 on alcohol intake. P rats were injected IP with vehicle or different doses of the selective D_2 antagonist S(-)-Eticlopride or the selective D_1 antagonist R(+)-SCH-23390 and 20 min. later with a dose of 0.75 mg/kg TA-0910 or vehicle. Alcohol and water intake were measured every two hr. up to six hr. and then at 24 hr. Food intake was measured at 24 hr. Neither S(-)-Eticlopride nor R(+)-SCH-23390 altered alcohol intake alone, however, S(-)-Eticlopride, but not R(+)-SCH-23390, dose-dependently diminished the suppressive effect of TA-0910 on alcohol intake in P rats. These data suggest involvement of D_2 receptors in the attenuating effect of TA-0910 on alcohol consumption (Supported in part by NIAAA Grant AA07809).

666.15

EFFECTS OF CHRONIC ALCOHOL CONSUMPTION AND AGING ON DOPAMINE, SEROTONIN AND METABOLITE LEVELS.

M.J. Druse-Manteuffel* and J. M. Woods. Mol. Cell. Biochemistry Dept., Loyola U Chicago, Stritch Sch. of Med., Maywood, IL 60153

The present study investigated the combined effects of chronic alcohol consumption and aging on the concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA). These monoamines and metabolites were quantitated in nigrostriatal and mesocorticolimbic brain areas of 5-, 14- and 24-month-old male Fischer 344 rats.

The results of these experiments demonstrated that there were both significant age- and alcohol-related changes in mesocorticolimbic brain areas. There was a significant age-associated decline of dopamine, and an alcohol-associated reduction of 5-HT and 5-HIAA in the ventral tegmental area. In contrast, there was an age-related increase of 5-HT in the ventral pallidum (VP) and of 5-HIAA in the frontal cortex, striatum, nucleus accumbens and VP. The ratio of 5-HIAA/5-HT was increased in aged rats in several brain areas. The noted dopaminergic and serotonergic abnormalities are of particular interest in light of the involvement of these neurotransmitter systems in alcohol use and abuse.

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666.16

STRAIN-DEPENDENT SUPPRESSANT EFFECTS OF DOPAMINERGIC, SEROTONERGIC AND OPIOIDERGIC AGENTS ON ALCOHOL INTAKE.

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Several strains of rats are known to consume large amounts of alcohol voluntarily, including the Fawn-Hooded (FH) rats and the selectively bred alcohol-preferring (P) and alcohol-accepting (AA) rats. Neurochemical differences have been reported for these strains, so it was predicted that they might respond differently to agents interacting with neurotransmitter systems known to modulate alcohol intake. Adult male FH, P, and AA rats were obtained from their respective breeding colonies in Chapel Hill, Indianapolis, and Helsinki and housed individually in wire mesh cages under standard laboratory conditions. The rats had free, continous access to food, water, and alcohol (10%, v/v). After establishment of stable baselines, rats were injected subcutaneously with either the dopamine agonist bromocriptine (5 mg/kg), the serotonin-1A agonist 8-OH-DPAT (0.125 mg/kg), the serotonin releaser fenfluramine (0.25-1.0 mg/kg), the opiate antagonist naltrexone (3-9 mg/kg), or vehicle at 4-day intervals. The P rats exhibited a significantly greater suppression of alcohol intake after bromocriptine, while the FH rats exhibited a significantly greater suppression after 8-OH-DPAT. In contrast, the AA rats were comparatively resistant to each compound. These findings indicate that alcohol-preferring rat strains are differentially sensitive to several drugs which suppress alcohol intake, making it unlikely that a single pharmacological treatment will be beneficial in all human alcoholics.

666.17

ETHANOL-INDUCED CHANGES IN ACTIVITY OF ALCOHOL-PREFERRING (P) & NONPREFERRING (NP) RATS: EFFECTS OF DOPAMINE (DA) AND 5-HT RECEPTOR ANTAGONISTS.

M.J. Lewis* T. Smith, J. Bryant, M. J. Cichelli and H.L. June. Neurobehavior Lab, Dept of Psych, Temple Univ. Phila. PA 19119/Howard University, Wash., D.C. 20059 & IUPUI, Ind., IN 46202

P rats, in comparison to NP and untrained randomly bred rats, orally self-administer ethanol (E) at high concentrations (>10%) and increase locomotor activity after parenteral and oral E. The present study examined the effects of intraperitoneal (IP) E on open field activity in both P and NP rats. Animals were injected with saline or E (0.25-1.0 gm/kg) and open field (Digiscan) activity was measured at 10 and 60 min. Animals then were injected with the DA antagonist haloperidol (0.1 - 0.5 mg/kg, ip), the 5-HT_3 antagonist MDL72222 (0.5 - 2.0 mg/kg ip), or saline prior to measurement of activity. NP rats showed lower activity at all E doses. P rats showed increased activity after low doses of E at 10 min. and depression at higher doses. Both pimozide and MDL72222 blocked the increase in activity in P rats. Both antagonists increased the depressant effects of E in both lines. These data support the putative role of dopamine and 5-HT_3 receptor mechanisms in E effects on activity.

(Supported in part by AA06263 & RR08016 and Temple University)

666.18

INCREASES IN VOLUNTARY ALCOHOL CONSUMPTION IN RATS FOLLOWING A SINGLE LARGE DOSE OF DMI.

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In the course of a series of studies to examine the relationship between lesions of the prefrontal cortex (PFC) in Wistar rats and voluntary alcohol consumption, we report the following phenomenon. Male Wistar rats were subjected to either bilateral aspiration lesions of the PFC, injections of 5,-7-Dihydroxy-tryptamine (5,7DHT) or sham lesions. The 5,7DHT treatment required pre-treatment with 25 mg/kg i.p. desmethyl-imipramine (DMI), a catecholamine uptake blocker that is commonly given to enhance the 5,7DHT exposure to serotonergic neurons The controls for the aspiration lesion group were not given DMI. All lesions and pre-treatments were given during deep anesthesia induced by 65 mg/kg i.p. pentobarbital. After the lesions, rats were placed on a 6-week, modified sucrose-fading procedure during which they were exposed to increasing concentrations of alcohol-containing solutions. At each concentration of sucrose (Suc) and ethanol (ETOH) including 10% Suc: 5%ETOH, 15% Suc: 5% ETOH, 3% Suc: 5% ETOH and 5% Suc: 10% ETOH the control rats that had received the DMI drank significantly more ethanol (in g/kg body weight) than the control group without DMI. The incresed drinking was not generalized since the DMI group drank less than the controls without DMI when a 20% sucrose alone solution was tested. The rats had food and tap water available at all times. An unusual aspect of the finding is that the increased consumption of ethanol solutions lasted throughout the length of the study. Following the final solution presentation, all animals were decapitated and the brains rapidly dissected. Brain regions are analyzed for norepinephrine, dopamine and serotonin and their metabolites to determine whether the single dose of DMI produced a measurable change in transmitter level. (supported by NIAA P50-AA3510)

666.19

ACUTE SUPPRESSION OF ETHANOL WITHDRAWAL SEIZURES AND CHRONIC ATTENUATION OF ETHANOL INTAKE BY AMPHETAMINE AND FENFLURAMINE COMBINATION.

L.Yu, H.Fisher, and G.C.Wagner*. Depts. of Psychology and Nutritional Sciences, Rutgers Univ., New Brunswick, NJ 08903.

Rats were rendered physically dependent on ethanol by semivoluntary consumption of a 4.5% ethanol-containing liquid diet. Approximately 60% of ethanol-dependent rats underwent audio-stimulation-induced seizures nine hours after ethanol was withdrawn. Rats exhibiting seizures had higher dopamine and lower serotonin levels in the striatum compared to rats not exhibiting seizures. Treatment with amphetamine (2 mg/kg) plus fenfluramine (8 mg/kg) in combination resulted in a significantly lower seizure rate (about 18%) in ethanol-withdrawn rats. The dependent rats treated with amphetamine plus fenfluramine and not exhibiting seizures had striatal dopamine and serotonin concentrations in parallel with control diet rats and dependent rats not exhibiting seizures. Thus, the amphetamine plus fenfluramine combination seemed to exert seizure-suppressing effects by decreasing striatal dopamine while increasing serotonin levels. Finally, this same treatment of amphetamine (2 mg/kg) plus fenfluramine (8 mg/kg), administered chronically, reduced consumption of a 5% ethanol solution while sparing the water intake during two-bottle choice tests in both ethanol-dependent and -nondependent rats. This drug combination may be beneficial for both the treatment of ethanol consumption and withdrawal.

666.20

ETHANOL/SUCROSE AND SUCROSE REINFORCEMENT IN MULTIPLE SCHEDULE COMPONENTS: EFFECTS OF AMPHETAMINE AND HALOPERIDOL.

C. Slawecki, C.W. Hodge and H.H. Samson*. The Neuroscience Program and the Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157-1083

It is of interest to examine whether ethanol-reinforcement involves the same neurobiological control mechanisms as are other reinforcers. A multiple schedule may provide advantages over concurrent schedules when exploring this question. Because multiple schedules limit responding to the duration of each component, the actions of various agonists and antagonists on behavior maintained by different reinforcers can be evaluated at similar times periods of drug action.

Six, male Long-Evans rats were trained to lever press during a 30 min daily session for a 5% sucrose/10% ethanol mixture presented in one 2 min component and 5% sucrose presented in the other 2 min component of a Multiple FR4 FR4 schedule. When responding was stable, dose effect curves were determined twice for amphetamine (0.0, 0.1, 0.3, 1.0 and 3.0 mg/kg) and then twice for haloperidol (0.0, 0.1, 0.25, 0.5 and 1.0 mg/kg). Responding in the sucrose/ethanol components was approximately 50% greater than during sucrose components. Amphetamine, at 1.0 mg/kg or greater reduced responding in both components, with a greater effect during the second determination. Haloperidol had an equal effect on responding in both schedule components, reducing responding at 0.3 mg/kg or greater. Tolerance to haloperidol was observed on the second determination of the dose effect curve. Dopaminergic processes appear to be involved for both reinforcers and tolerance to the antagonist resulted from multiple injections.

666.21

INVOLVEMENT OF CENTRAL BUT NOT PERIPHERAL NICOTINIC ACETYLCHOLINE RECEPTORS IN THE DOPAMINE ACTIVATING AND REINFORCING EFFECTS OF ETHANOL. Q. Blomqvist*, J.A. Engel and B. Söderpalm. Inst. of Physiology and Pharmacology, Dept. of Pharmacology, Göteborg University, Medicinaregatan 7, S-413 90 Göteborg, Sweden.

We have previously shown that mecamylamine (1.0 mg/kg i.p.), a blood brain barrier penetrating antagonist at the nicotinic acetylcholine receptor (nAChR), completely blocks ethanol-induced (2.5 g/kg i.p.) dopamine (DA) overflow in the nucleus accumbens in Wistar rats, as measured by *in vivo* microdialysis. Mecamylamine also decreased voluntary ethanol intake in high-preferring but not low-preferring rats, classified after a pre-experimental screening period in a free choice situation between an ethanol solution (6% v/v) and water. Hence, we hypothesized that ethanol's DA releasing effect, which may be of importance for its reinforcing effect, is mediated via activation of central nAChR, perhaps through a direct ethanol-nAChR interaction. Involvement of peripheral nAChR could, however, not be excluded.

In the present study, hexamethonium, a nAChR antagonist which does not penetrate the blood brain barrier was used in order to elucidate the possible involvement of peripheral nAChR in the previous findings. Hexamethonium-chloride was administered (10 mg/kg i.p.) 20 minutes prior to ethanol (2.5 g/kg i.p.), and DA overflow in the rat nucleus accumbens was measured by *in vivo* microdialysis. Although dialysis probe insertion might cause a discrete blood brain barrier defect resulting in hexamethonium diffusion into the brain, no antagonism of ethanol-induced DA overflow was observed. Furthermore, hexamethonium treatment did not alter ethanol drinking behavior in high-preferring or low-preferring rats. In conclusion, unless mecamylamine and hexamethonium display significant pharmacodynamic differences in addition to their pharmacokinetic differences, peripheral nAChR are not likely involved in the DA activating and reinforcing effects of ethanol.

PSYCHOTHERAPEUTIC DRUGS: ANTIPSYCHOTICS II

667.1

ANTIPSYCHOTIC DRUGS' MECHANISMS OF ACTION: INTERACTIONS AT THE NMDA RECEPTOR. T.J.Lidsky*, E.Yablonsky-Alter, L.Zuck, S.P. Banerjee. Inst. for Basic Research, Staten Island, New York 10314.

Glutamatergic dysfunctions have long been implicated in psychosis. Based on this consideration, the present study of antipsychotic drug interactions with the NMDA receptor was undertaken. In cortical tissue extensively washed to reduce residual levels of glutamate and glycine, [^{3}H]MK-801 binding is minimal. However, addition of either ligand at non-saturating concentrations increases ionophore opening resulting in a two- to threefold enhancement of [^{3}H]MK-801 binding and is thus commonly used as a functional assay of NMDA activity. Addition of either chlorpromazine, thioridazine, haloperidol or clozapine all further increased the already elevated [^{3}H]MK-801 binding caused by either glutamate and/or glycine. This effect was prominent at drug concentrations similar to that found in schizophrenic patients' plasma water or CSF when antipsychotic efficacy is achieved. At higher concentrations inhibition of NMDA activity was seen for all drugs. Augmentation at low and inhibition at higher concentrations was confirmed in electrophysiogical studies of glutamatergic transmission in the striatal slice preparation. These data, consistent with partial agonism, suggest that a unique action at glutamate synapses contributes to antipsychotic efficacy and further implicate glutamatergic dysfunction in the etiology of schizophrenia.

667.2

CHANGES IN STARTLE, EVOKED POTENTIALS, SPEM, SUSTAINED ATTENTION AND NEGATIVE SYMPTOMS FRAME A "THERAPEUTIC WINDOW" FOR NEUROLEPTICS K.A. Young*, M.D. Brady, C. Poche and P.B. Hicks. Department of Psychiatry and Behavioral Science, Texas A&M University Health Science Center, Scott & White Clinic and the Waco VAMC Neuroscience Laboratory, Waco, Texas 76711.

The influences of different doses of chronic neuroleptic medication on multiple information processing behaviors and on clinical symptoms were determined in long-term schizophrenic subjects with a cross-sectional design. A multidimensional information processing battery and standard CPT and WCST exams were administered in a one hour session. Inverted U-shaped neuroleptic dose-response curves were observed for startle prepulse inhibition deficit, N100/P200 evoked potential gating deficit and P200 evoked potential latency. U-shaped curves with minima corresponding to the peaks of the inverted curves were observed for negative symptoms and PANSS symptoms of anergia. Similarly shaped curves may also be present for the Continuous Performance Task, smooth pursuit eye movement and delta EEG activity. Dose-related changes in positive symptoms occurred only at low chronic neuroleptic doses. The dose-related correlation of low levels of negative symptoms and maximal changes in information processing suggestive of dopaminergic hyperactivity provides indirect evidence that moderate doses of neuroleptics may have an action to increase the availability of forebrain dopamine. The low dose suppression of positive symptoms, and the high dose reversal of moderate dose improvements in negative symptoms lends support to the concept that classical neuroleptics have a "therapeutic window" that is framed by positive symptom remediation at low doses and deterioration of negative symptoms and information processing behaviors at high doses.

667.3

CLOZAPINE INCREASES EXTRACELLULAR DOPAMINE IN THE RHESUS MONKEY PREFRONTAL CORTEX. K.D. Youngren*, F.M. Inglis, H.P. Jedema, P.J. Pivirotto, J. Bagley, C.W. Bradberry, B.S. Bunney, P.S. Goldman-Rakic, R.H. Roth and B. Moghaddam. Neuroscience Program, Depts. of Psychiatry and Pharmacology, and Sect. of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510, and VA Med. Center, West Haven, CT 06516.

The effects of clozapine and haloperidol on extracellular levels of dopamine were examined in cortical and subcortical regions of the rhesus monkey brain using intracerebral microdialysis. MRI-directed stereotaxic surgery was used to implant guide cannulae in three young adult male animals. Microdialysis probes were lowered into discrete brain regions through the guide cannulae while animals were under isoflurane gas anesthesia. Several dopamine-terminal-containing cortical regions were examined. These include: dorsolateral prefrontal cortex (Brodmann's area 46), medial prefrontal cortex (areas 8 and 9), and premotor cortex (area 6). Subcortical regions examined include the caudate and putamen. Basal extracellular levels of dopamine were reliably measured in all of these discrete brain regions. A stable baseline in extracellular dopamine was established for a minimum of one hour before drug administration. Clozapine (2 mg/kg i.v.) produced a significant increase in extracellular levels of dopamine in all brain regions examined, both cortical and subcortical. Haloperidol (0.2 mg/kg i.v.), in contrast, produced little or no change in extracellular dopamine levels in cortical regions or in the caudate. These results provide evidence in the non-human primate that acute administration of the atypical antipsychotic drug clozapine exerts a potent effect on cortical dopamine systems in comparison to the typical drug haloperidol. Supported in part by USPHS Award MH-44866.

667.4

MODIFICATION OF CLOZAPINE-ELICITED FOS EXPRESSION IN THE PREFRONTAL CORTEX AFTER BASOLATERAL AMYGDALA LESIONS. D. S. Cameron*, C. D. Young, and A. Y. Deutch. Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508 and the VA Medical Center, West Haven, CT 06516.

Administration to rats of the atypical antipsychotic drug clozapine results in a marked increase in the expression of the immediate-early gene c-*fos* or its protein product Fos in the medial prefrontal cortex (PFC). Administration of other antipsychotic drugs (APDs) does not induce Fos expression in the PFC. The mechanisms underlying the selective effect of clozapine on PFC Fos expression is not well understood, but D_2-like, D_1-like, 5-$HT_{2a/2c}$, α_1, and muscarinic cholinergic receptors do not appear to be critical factors. We attempted to determine if lesions of key afferents to the PFC modify the cortical Fos response to clozapine. Lesions of the thalamic paraventricular nucleus, which projects to the deep layers of the infralimbic and prelimbic cortices, did not modify the clozapine-elicited increase in PFC Fos expression, as assessed by immunoblots. However, lesions directed at the basolateral nucleus of the amygdala enhanced Fos expression in the PFC in response to clozapine challenge. The mechanisms subserving the modification of the cortical Fos response to clozapine are currently being examined. Supported in part by MH-45124, the VA Schizophrenia and Post-Traumatic Disorder Research Centers, and the National Parkinson Foundation Center of Excellence at Yale University.

667.5

DISCRIMINATIVE STIMULUS PROFILE OF CLOZAPINE, OLANZAPINE AND CHLORPROMAZINE IN MIANSERIN AND SCOPOLAMINE TRAINED RATS. B. M. Kelley, J. H. Porter* and S. A. Varvel. Dept. of Psychology, Virginia Commonwealth University, Richmond, VA 23184-2018.

The atypical antipsychotic clozapine (CLZ), the typical antipsychotic chlorpromazine (CPZ), and the putative atypical antipsychotic olanzapine (OLZ) were tested in rats trained to discriminate the serotonergic (5-HT) antagonist mianserin (MIA, 4.0 mg/kg) from saline and in rats trained to discriminate the muscarinic antagonist scopolamine (SCP, 0.25 mg/kg) from saline. CLZ produced full substitution in the MIA-trained rats with 87.5% drug-lever responding (DLR, 1.25 mg/kg dose) and partial substitution in the SCP-trained rats with 60.9% DLR (5.0 mg/kg dose). The 2.0 mg/kg dose of CPZ produced partial substitution in the MIA-trained rats (58.5% DLR), but did not substitute for SCP (18.9% DLR). OLZ produced a substitution profile intermediate between CLZ and CPZ. The 1.25 mg/kg dose of OLZ produced 66.8% DLR in the MIA-trained rats and 36.0% DLR in the SCP-trained rats.

The selective 5-$HT_{2A/2C}$ antagonist ritanserin produced complete substitution in the MIA-trained rats (98.4% DLR, 5.0 mg/kg dose) but did not substitute for SCP (25.0% DLR, 2.5 mg/kg dose). Interestingly, there was an asymmetrical cross-generalization between MIA and SCP. While MIA failed to produce SCP-appropriate responding,SCP produced 88.0% DLR in the MIA-trained rats. The present results demonstrated that the two-lever drug discrimination paradigm reveals different profiles for the atypical antipsychotic clozapine and the typical antipsychotic chlorpromazine in both MIA- and SCP-trained rats. The putative atypical drug olanzapine produced a discriminative stimulus profile intermediate between that of CLZ and CPZ. Testing with other typical and putative atypical antipsychotics will be necessary to establish the predictive validity of this behavioral screen for atypical antipsychotics.

667.6

THE INFLUENCE OF HALOPERIDOL ON THE PEAK VELOCITY OF HORIZONTAL AND VERTICAL SACCADES. B. Guldin , J. Heermann and W. Poewe (Spon: European Neuroscience Association). Virchow-Klinikum der Humboldt-Universität, Neurologie, 13353 Berlin, Germany.

Metoclopramide and haloperidol may cause oculogyric crises in susceptible patients. We were interested in their influence on horizontal and vertical saccades in order to determine the qualitative or quantitative nature of such crises.In a pilot study (open design) three healthy male volunteers (23-28 years old) received metoclo-pramide 10 mg tid. Eye movement registration with infrared oculography was done before the application of the test substance, seven hours, and 22 hours after the first dose (six hours past the third dose). In two of the subjects the horizontal and vertical saccades were all slower at the third measurement compared to baseline. In the second pilot study five healthy male volunteers received haloperidol 2 mg tid in a double-blind placebo-controlled design. The protocol of eye movement registration is described above. Four of the volunteers had slower saccadic peak velocities for horizontal and vertical saccades after the ingestion of haloperidol compared to placebo.
D2-antagonists probably decrease the peak velocity of saccades unspecifically through their sedating effects. Regarding the oculogyric crises our suggestion is that there is no specific path of action of d2-antagonists on vertical eye movements and that the tendency of some patients to develop oculogyric crises on these drugs is due to differences in density and proportion of receptor-subtypes.

667.7

ATYPICAL BEHAVIORAL EFFECTS OF CHRONIC CLOZAPINE TREATMENT IN THE RAT ARE NOT ELIMINATED BY SEROTONIN DEPLETION G.E. Jaskiw*, B. Yrtunski Psychiatry Service, Cleveland VAMC (541), Brecksville, OH 44141 and Dept of Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

Rats withdrawn from chronic treatment with clozapine (CLOZ) (in contrast to haloperidol (HAL)) do not show an augmentation of higher order stereotypies (gnawing, biting) after apomorphine (APO) challenge. It has been postulated that some of CLOZ's atypical effects are mediated via serotonin (5HT) systems. To test the latter, 5HT depletion was achieved in rats by infusing 5,7-dihydroxytryptamine icv, after pretreatment with nomifensine and desipramine. After a 28d recovery period, groups of SHAM and 5HT depleted animals were assigned to a 21d course of treatment with VEH, HAL 1 mg/kg/d or CLOZ 20 mg/kg/d in drinking water. After a 3d withdrawal period, animals were challenged with APO 0.75 mg/kg SC and monitored for 90 min. Brain regions were assayed for monoamines. As anticipated HAL but not CLOZ pretreated animals showed an increase in biting behaviors and a reduction in time spent being stationary. Differences between HAL and CLOZ treated rats persisted despite a high and selective degree of 5HT depletion. Our data suggest that at least some of CLOZ's preferential actions on mesolimbic DA systems do not depend on an intact central 5HT system. Supported by VAMC grant.

667.8

REFLEX COMPONENTS IN WRIST ROTATION OF CLOZAPINE-TREATED SCHIZOPHRENICS. P.B. Vrtunski*, P.E. Konicki, G.E.Jaskiw, M.Simon and K.Y. Kwon. Cleveland VAMC and Department of Psychiatry, CWRU School of Medicine, Cleveland, OH 44141.

Several reports have suggested that the atypical antipsychotic drug clozapine (CLOZ) may have an adverse effect on various motor tasks including eye-movements, reaction time response or submaximal force maintenance. In all of these studies, tasks relied on voluntary movements and considerable attentional effort on the part of the subject. The intent of this study was to establish whether similar clozapine-induced deficits in motor control can be observed in outputs with minimized voluntary, and maximized unpredictable aspects of response. Ten chronic schizophrenics, tested before and after initiation of clozapine treatment, were compared to seven normal controls. The method included subject's holding a handle in vertical position and "matching" a target light with the handle-position light. At random intervals, supinating or pronating stimuli, 0.5 s long, of two different intensities, were applied to the handle. Handle movement was controlled by a torque motor and its position was read by an optical (5 pulses/degree) encoder. Resistance to handle movement was obtained from tracings during torque-stimuli (time-integral-of-force). Three findings were made: (a) Reflex response to handle movement readily discriminates normal subjects from those with schizophrenia (most likely due to medication induced rigidity). (b) Initiation of clozapine does not abolish the observed rigidity. (c) Clozapine attenuates resistance to torque-stimuli several-fold ($F(1,6) = 16.75$, $p < 0.01$). We conclude that reflex response to kinetic stimuli may provide important information regarding the motor mechanisms and antipsychotic medication. (Supported by the Veterans Administration and USPHS grant MH-46630)

667.9

CLOZAPINE AND FORCE CONTROL DECLINE AT TREATMENT INITIATION OF SCHIZOPHRENICS. P.E. Konicki*, G.E. Jaskiw, K.Y.Kwon, M.Simon and P.B. Vrtunski. Cleveland VAMC and Department of Psychiatry, CWRU School of Medicine, Cleveland, OH 44141.

In evaluating the effects of antipsychotic drugs on force control (FC) we recently demonstrated that clozapine (CLOZ) treatment is associated with a reduction in voluntary movement control; this effect did not appear to be related to pre-existing patient characteristics, but rather to clozapine treatment *per se* (Soc.Neurosci.Abstr., 20: 854, 1994; submitted). Since CLOZ treatment is usually initiated by a titration from 12.5 mg/d to about 450 mg/d over 3-4 weeks, we now present a study of the relationship between CLOZ dose and the decline in force control. The FC task consisted of 42 10-second long trials in which the patient was instructed to match a target light with a response light. The response consisted of patients' index-finger pressure on a force-sensitive button. There were 7 targets of increasing force (20 to 480 centiNewtons), each presented 6 times. The steadiness of force maintenance was the dependent variable. In seven cases studied so far, force control variability increased in all patients, from the pre-treatment mean of 2.51 (±0.53) to 3.10 (±0.69) reached at the mean daily dose of 410 mg/day. The paired t-test indicated the difference to be significant ($t = 3.40$, $p < 0.02$). The dose - response relationship, however, was not linear: in two patients the FC decline was a positively accelerated function of dose increase; in another patient a maximum FC decline was recorded at lower dose (150 mg/day), probably due to a strong sedative effect. We conclude that force control analysis may be useful both for establishing dose-response function of clozapine, and for more precise understanding of individual differences in response to this antipsychotic agent. (Supported by the Veterans Administration and USPHS grant MH-46630)

667.10

THE EFFECT OF CLOZAPINE ON FOS PROTEIN IMMUNOREACTIVITY IS NOT MIMICKED BY THE ADDITION OF α1-ADRENERGIC OR 5HT RECEPTOR BLOCKADE TO HALOPERIDOL. T. Specht Ludvigsen, Niels Korsgaard, Peter Kristensen* and A. Fink-Jensen. Health Care Discovery, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark

The involvement of α1-adrenergic or 5HT2-receptor blockade in the atypical profile of clozapine was investigated in the rat by use of Fos protein immunoreactivity, a technique, which has been used to identify the anatomical substrate underlying the effects of typical and atypical neuroleptics. Clozapine (20 mg/kg) induced a significantly higher Fos protein immunoreactivity response in the prefrontal cortex and significantly lower response in the dorsolateral striatum compared to the effect of haloperidol (1 mg/kg). Prazosin (0.3 and 1 mg/kg) and ritanserin (1 and 3 mg/kg) did not increase Fos protein immunoreactivity by themselves and did not mimic the clozapine response when co-administered with haloperidol (1mg/kg). Consequently, the present study does not suggest, that the α1-adrenergic receptor blockade or the 5HT2-receptor blockade exclusively accounts for the atypical profile of clozapine compared to haloperidol.

667.11

LOW-DOSE SLOWING OF RATS' LAPPING RHYTHM AS A POTENTIAL MARKER FOR ATYPICAL NEUROLEPTICS. S.C. Fowler* and S. Das. Dept. of Human Development, Univ. of Kansas, Lawrence, KS 66045.

The acute behavioral effects of the atypical neuroleptic clozapine (0.5-4.0 mg/kg), were compared with those of olanzapine (0.25-2.0 mg/kg) and risperidone (0.04-0.08 mg/kg). As thirsty rats (n=29) lapped water from a force-sensing disk, the force of tongue contact was continuously recorded and then Fourier analyzed to yield estimates of the lick rhythm (in Hertz). All three drugs significantly, and dose dependently reduced the lick rhythm, and the lowest dose of each drug had this effect even when the total number of licks per 2-min session was not significantly reduced. Neither the typical neuroleptic haloperidol nor the selective D2 dopamine receptor antagonist raclopride produced this slowing effect. The doses producing half the maximal effect on rhythm slowing for risperidone, olanzapine, and clozapine were 0.06, 0.55, and 1.16 mg/kg, respectively. The rank order of potencies suggested a serotonin-2 receptor involvement in the slowing of lick rhythm. Supported by MH43429.

667.12

ATYPICAL PROFILE OF THE ANTIPSYCHOTIC CANDIDATE BW1205U90 IN A PRIMATE SOCIAL COLONY MODEL FOR SCREENING ANTIPSYCHOTIC AGENTS. R.F. Schlemmer*, J.E. Young, C.K. Fleming, D.J. McGinness-Grimes, and J.M. Davis. University of Illinois at Chicago, Chicago, IL 60612.

BW1205U90, ((+/-)-cis-2-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl)-4A,5,6,7,8,8A-hexahydro-1(2H)-phthalazinone HCl), has been identified as a potential atypical antipsychotic drug (AP) (Rigdon and Norman, Neurosci.Abs. 20:1639, 1994). It binds to 5-HT_2 sites with greater affinity than to D_2 and has appreciable affinity for the 5-HT_{1A} and α_1-NE sites. This study assessed the behavioral effects of BW1205 alone and with apomorphine (Apo) in a non-human primate screening model for AP. Four females from a colony of 5 stumptail macaques (Macaca arctoides) were assigned to 2 treatment groups of 2 females each. After determining baseline behavior, each group received drug treatment for 2 days/week in a cross-over design. First, Apo was given alone, 1 mg/kg, i.m. at 1045 for 2 days. Then, 5 escalating doses of BW1205, 0.1-3.0 mg/kg n.g. were given at 0800 and 1630 on Day 1 and at 0800 on Day 2. Two 1 hr observation sessions were conducted at 0930 and 1100 each day to observe the effects of BW1205 alone and with Apo respectively. On Day 2 of treatment BW1205 produced a dose-dependent antagonism of the Apo-induced increase in submissive gestures given by treated monkeys and checking (visual scanning). However, BW1205 failed to robustly antagonize Apo-induced stereotypy and produced only low levels of movement abnormalities at doses $\geq$ 1 mg/kg when given alone. Antagonism of increased submissiveness and checking is similar to known AP. The more than 10-fold separation between antagonist dose vs. movement disturbance threshold dose and weak antagonism of stereotypy is comparable to clozapine. Thus, the behavioral profile of BW1205 in primates is consistent with that of an atypical AP. (Funded by a gift from Burroughs-Wellcome, Research Triangle Park, NC).

667.13

DOPAMINE D3 RECEPTORS MEDIATE CLOZAPINE-INDUCED C-FOS EXPRESSION IN THE FOREBRAIN. N. Guo*, S.R. Vincent and H.C. Fibiger. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1Z3

The atypical antipsychotic clozapine produces a unique pattern of c-*fos* expression in the forebrain. While the receptor mechanisms by which this occurs are not well understood, there is some evidence to suggest that D2-like receptors may be involved. To determine if D3 receptors contribute to clozapine's effects on regional c-*fos* expression, in situ hybridization histochemistry with a cDNA oligonucleotide probe was used to identify cells that synthesize D3 receptor mRNA.

Consistent with previous studies, D3 receptor mRNA was enriched in the islands of Calleja (IC). In addition, a moderate density of D3 mRNA signal was observed in the lateral septum (LS). Fos-immunostaining combined with in situ hybridization histochemistry demonstrated that most clozapine-induced Fos-positive neurons in the IC express mRNA encoding D3 receptors, and that the majority of Fos-positive neurons in the LS and nucleus accumbens (NAc) contain D3 receptor mRNA. Further studies with oligonucleotide probes for enkephalin and dynorphin mRNA demonstrated that in the NAc and LS, some Fos-positive neurons express enkephalin mRNA and some contain dynorphin mRNA. In the IC, many Fos-positive neurons express dynorphin while very few express enkephalin mRNA.

A previous study has demonstrated that clozapine-induced c-*fos* expression in the NAc, IC and LS is blocked by D3 receptor agonists, suggesting that clozapine's effects in these regions are due to its antagonist actions at D3 receptors (Neurosci. 65, 3: 747). The co-localization of D3 receptor mRNA and Fos protein following clozapine administration in limbic brain neurons is consistent with this hypothesis.

667.14

MUSCARINIC m4 RECEPTOR AGONISM BY ATYPICAL BUT NOT TYPICAL ANTIPSYCHOTICS. X.P. Zeng , F. Le, I. Scarisbrick, and E. Richelson. Neuropharmacology Laboratory, Mayo Clinic Jacksonville, Jacksonville, FL 32224

The muscarinic m4 receptor agonist effects of clozapine as recently reported by Zorn et al., 1994, may be related to its therapeutic and side effects as an antipsychotic drug (Tandon & Kane, 1993). We have further examined the effect of a group of neuroleptics on forskolin-stimulated cyclic AMP accumulation in Chinese hamster ovary cells (CHO) expressing human m4 receptors to determine the possible relationship between m4 agonism and their pharmacological efficacy. Comparable to carbachol, at 1 μM, all the atypical neuroleptics tested - clozapine, olanzapine, fluperlapine, rilapine and tenilapine - caused a 20 to 50% inhibition of forskolin (500 μM)-stimulated increase of cAMP. These effects were totally reversed by pretreatment with 10 μM atropine. In contrast, a series of typical neuroleptics - chlopromazine, flupherazine, molindone and thiothixene - did not show the inhibition. In addition, none of the drugs tested caused a significant change in the basal concentration of cAMP. Therefore, m4 receptor agonism appears to be a common mechanism for atypical antipsychotic drugs, which may explain differences in the therapeutic efficacy and side effects between atypical and typical drugs. (Supported by Mayo Fdn. And USPHS Grant MH27692)

Tandon, R. & Kane, J.M. Arch. Gen. Psychiatry 50:158, 1993.

Zorn, S.H. et al. Eur.J.Pharmacol. 269:R1-2, 1994.

667.15

CLOZAPINE (CLOZ) PLUS SKF38393 STIMULATES THE ACTIVITY OF DOPAMINE (DA)-DEPLETED RATS. D. M. Jackson, N. Mohell, A. Bengtsson, C. Wallsten* and Å. Malmberg. Depts of Behavioural and Biochemical Pharmacology, and Molecular Pharmacology, Astra Arcus AB, Preclinical R & D, S-151 85, Södertälje, Sweden.

The aim of the present experiments was to investigate the locomotor stimulant effects of the atypical antipsychotic agent CLOZ in rats depleted of their DA by reserpine and α-methyl-p-tyrosine pretreatment. CLOZ itself induced a slight but non-significant activation which was enhanced by the addition of the selective D_1 agonist SKF38393 but not by the selective D_2 agonist quinpirole. The activation produced by CLOZ plus SKF38393 was partially blocked by the D_1 antagonist SCH23390, while the D_2 antagonist, haloperidol, was ineffective. A combination of SCH23390 and haloperidol completely blocked the CLOZ plus SKF38393 induced locomotion. Unlike the effect seen with CLOZ, neither the 5-HT_2 antagonist ritanserin nor the D_2 antagonists haloperidol and remoxipride caused locomotor activation when given alone or in combination with SK-F38393. d-Amphetamine was inactive in the monoamine depleted rats indicating that no DA was available for release by d-amphetamine. The muscarinic antagonist scopolamine was inactive, but produced marked stimulation when combined with SKF38393 but not with quinpirole. This stimulation was not affected by haloperidol, nor was it completely blocked by SCH23390 alone or by the combination of haloperidol and SCH23390. The data indicate that CLOZ, in rats depleted of their DA stores, exhibits properties like those of a DA agonist. The pharmacology of this behavioural stimulation was similar, but not identical, to that seen with the muscarinic antagonist scopolamine. The DA agonist like behavioural effects of CLOZ reported here may therefore be due in part to a muscarinic receptor antagonist like effect.

667.16

ANTAGONISM OF PCP-INDUCED DEFICITS IN PREPULSE INHIBITION BY OLANZAPINE. V.P. Bakshi* and M.A. Geyer. Dept. of Neuroscience, UCSD, La Jolla, CA 92093.

Presentation of a weak stimulus (the prepulse) immediately prior to an intense startling stimulus (the pulse) results in an attenuation of the startle response to the pulse. This phenomenon of prepulse inhibition (PPI) is thought to be a measure of sensorimotor gating mechanisms, which are deficient in schizophrenia patients. PPI is disrupted in schizophrenia patients and in rats, PPI is disrupted by the psychotogenic noncompetitive NMDA antagonists phencyclidine (PCP) and dizocilpine (MK-801). Reversal of PCP- and MK-801-induced deficits in PPI is produced by the atypical antipsychotic clozapine, but not by traditional neuroleptics. Olanzapine is a putative atypical antipsychotic that shares many pharmacological and pre-clinical behavioral characteristics with clozapine. The purpose of the present investigation was to determine if olanzapine would also reverse PCP- and MK-801-induced disruptions in PPI. Rats were treated with either 1.25, 2.5, 5.0 or 10.0 mg/kg of olanzapine (i.p.) or saline and then treated with PCP (0 or 1.5 mg/kg, s.c.). In a second study, separate groups of rats were given either saline or olanzapine (10.0 mg/kg, i.p.) followed by MK-801 (0 or 0.1 mg/kg, s.c.). After drug administration, animals were placed into startle chambers. PCP and MK-801 dramatically reduced PPI. Olanzapine by itself did not affect PPI, but did antagonize both the PCP- and MK-801-induced deficits. Effects on PPI were independent of changes in baseline startle amplitude. Finally, olanzapine accelerated habituation of the startle response to repeated tactile stimuli. These results provide further evidence for the functional homogeneity of olanzapine and clozapine. Studies to determine the mechanisms underlying these effects are in progress.

667.17

INTRA - ACCUMBENS CLOZAPINE AND HALOPERIDOL ENHANCE LATENT INHIBITION IN RATS. L.A. Dunn* and R.J. Scibilia. Department of Psychiatry, Duke University Medical Center, Durham, North Carolina 27710.

Latent inhibition (LI) is an indirect behavioral measure of selective attention that is sensitive to dopaminergic and serotonergic receptor modulation. Haloperidol enhances LI across a broad dose range. Clozapine enhances LI only at very low doses (0.1 and 0.3 mg/kg) and reduces LI at moderate (3.0 mg/kg) doses (Dunn and Scibilia, Soc. Neurosci. Abstr., 20:1, 226, 1994). The anatomic locus of these effects is being explored. Sprague-Dawley rats weighing 150-175 grams were fitted with bilateral chronically indwelling canulae located just above either the nucleus accumbens (coordinates from bregma: A=2.2 mm, L=±1.5 mm, V=-7.2 mm, head 5° above intraaural line), or the amygdala (coordinates from bregma: P=-0.3mm, L=±4.3 mm, V=-8.1mm, head 5° above intraaural line). Six days following surgery rats were started in a LI measurement procedure published previously (Dunn et al., 1993, Psychopharmacology, 112:315-323). This consisted of 4 days of lick training, 1 day of preexposure (20 or 0 light stimuli) and conditioning (2 light, shock pairings), and 1 day of testing. Haloperidol, 50 ng in 1 µl, or clozapine 1 µg in 1 µl was administered once 2 minutes prior to preexposure. Vehicle was 0.3% w/v tartaric acid pH adjusted to 6.8. Haloperidol enhanced LI in both the nucleus accumbens ($p<0.02$) and the amygdala ($p<0.025$). Clozapine enhanced LI in the nucleus accumbens ($p<0.05$), but reduced LI in the amygdala ($p<0.05$). The divergent effects of clozapine in these nuclei is likely due to a differential sensitivity to 5-HT2a/c antagonism. (Supported by NIMH #47503)

667.18

CONTRASTING EFFECTS OF CHRONIC CLOZAPINE, SEROQUEL AND HALOPERIDOL ADMINISTRATION ON ΔFosB EXPRESSION IN THE FOREBRAIN F. Ansari*[1], Y. Nakabeppu[2] and G.S. Robertson[1] Dept. of Pharmacology[1], University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5. Dept. of Biochemistry[2], Medical Institute of Bioregulation, Kuyshu University 69, Fukuoka 812, Japan.

We have recently demonstrated that specific neuroanatomical patterns of increased Fos-like immunoreactivity (FLI) are predictive of atypical antipsychotic activity. However, the fact that neuroleptics must be administered chronically in order to generate both extrapyramidal side effects (EPS) and an optimal therapeutic response calls into question the relevance of acute changes in FLI for these slowly developing events. In resolution of this problem, we have discovered that the immediate-early gene product ΔFosB is expressed in neurons activated by chronic alterations in dopaminergic neurotransmission. Chronic administration of typical and atypical neuroleptics may therefore produce distinct patterns of ΔFosB expression that can be used to identify such compounds and their neuronal targets. To test this hypothesis, we compared the effects of chronic haloperidol, clozapine and seroquel administration on ΔFosB-like immunoreactivity (ΔFosB-LI) in the rodent forebrain. Administration of haloperidol (2 mg/kg/day) for 19 days dramatically elevated ΔFosB-LI in both the dorsolateral and ventral striatum. In contrast, administration of either clozapine (20 mg/kg/day) or seroquel (10, 20 mg/kg) for 19 days elevated ΔFosB-LI primarily in limbic regions such as the prefrontal cortex, ventral striatum and lateral septal nucleus. These patterns of chronically increased ΔFosB-LI are similar to those observed acutely for FLI suggesting that ΔFosB may be a relevant maker for neuronal populations activated by prolonged neuroleptic administration.

AGING: PRIMATES INCLUDING HUMANS

668.1

VARYING THE TRACE INTERVAL IN EYEBLINK CONDITIONING IN YOUNG AND AGING HUMANS. M.C. Carrillo*, C.T. Fitzpatrick & J.F. Disterhoft. CM Biology, Northwestern University Med. Sch, Chicago, IL.

Eyeblink conditioning has been used extensively to investigate the neural substrates of associative learning. Recent studies have found the effects of age and interstimulus interval in delay conditioning to change over life span (Solomon, et al., 1991). Other studies of trace conditioning have found nonoptimal interstimulus intervals (Woodruff-Pak et al., 1991). Our goal was to determine an optimal trace interval in the eyeblink conditioning paradigm for young and aging subjects, and subsequently test medial temporal lobe amnesics and Korsakoff amnesics with the optimal interval.

Younger (n=32, 20-38 yrs) and older (n=32, 60-75 yrs) humans were randomly assigned to one of four trace intervals: 250, 500, 750, and 1000 ms. Eyeblink conditioning involved the presentation of an 85 db, 100 ms, 3 kHz tone, followed after a silent trace interval by a 100 ms, 3–6 psi corneal airpuff sufficient to elicit reliable unconditioned responses. Sessions consisted of 30 pseudoconditioning trials (unpaired tone and puff presentations), 60 conditioning trials, and 30 tone-alone extinction trials. A correction method was used to eliminate responses that could contaminate data, including voluntary or alpha responses and random blinks.

No aging difference in mean percent conditioned responses was observed for the 250 ms ISI, Young=32.6%, Aging=32.1%. Significant aging differences were found for the remaining three intervals: 500 Young=55.8%, Aging=29.3; 750 Young=34.6%, Aging=16%; 1000 Young=21.8%, Aging=10.7 ($p<.05$). These data suggest that the trace eyeblink conditioning interval which resulted in the highest conditioning performance for young and aging humans was 500 ms. Having an optimal trace interval for young, as well as aging subjects is relevant when testing patient populations which vary in age. Currently, medial temporal lobe and Korsakoff amnesics are being tested using the 500 ms trace eyeblink conditioning paradigm. Supported by AG 08796 to JFD, GM 17223-01 to MCC, RR-00048 to CRC NMH.

668.2

A COMPARISON OF CLASSICAL EYEBLINK AND HEART RATE CONDITIONING IN YOUNG AND OLD HUMAN SUBJECTS AND THIER RELATIONSHIP TO OTHER COGNITIVE TASKS. Samantha Walker, Janet Hunt, S.L. Buchanan* & D.A. Powell. VA Medical Center and the University of South Carolina, Columbia, SC 29208.

Young (28-35 yrs.) and old (60-80 yrs.) human subjects received a single session of Pavlovian conditioning using a 500 msec tone as the conditioned stimulus and a 100 msec corneal airpuff as the unconditioned stimulus. Eyeblink and heart rate conditioned responses were recorded. A number of other cognitive tests were also administered to determine the relationship between Pavlovian conditioned EB and HR responses and other kinds of cognitive tasks. Age-related deficits in both EB and HR conditioning occurred. In addition, it was found that various aspects of EB conditioning were correlated with performance on several declarative but not nondeclarative tasks. Significant correlations were obtained, for example, between total EB CRs and performance on paired associate and verbal recall tests. Both HR and EB conditioning were also correlated with scores on the WAIS vocabulary subtest. These correlations may reflect a declarative component to classical conditioning that is at least partially responsible for the large age-related deficits in classical conditioning found in this and other experiments.

Supported by VA Institutional Research Funds

668.3

MRI DETECTED CEREBELLAR ATROPHY DURING AGING. Michael P. Sullivan, Leyla deToledo-Morrell, Frank Morrell*, and Sandra Spencer. Departments of Neurological Sciences, Psychology and Diagnostic Radiology, Rush Medical College, Chicago, IL. 60612.

Classical, delay conditioning of the eyeblink response has been shown to be impaired in aged individuals, thus implicating cerebellar dysfunction. With the use of high resolution, quantitative, magnetic resonance imaging (MRI) protocols, it is now possible to detect and quantify, *in vivo*, the extent of age or disease induced alterations in given brain regions of interest. In the present study, the presence of cerebellar atrophy was assessed in healthy, aged individuals as part of a larger investigation designed to examine the effects of aging on brain anatomy *in vivo*. 37 aged (mean age=70, range=61-84) and 27 young (mean age=27, range=22-34) subjects were studied with a high resolution MRI protocol. An interactive, 3-D reconstruction program was used to compute the volume of regions of interest. Cerebellar volume was derived from 18 gapless, 5mm sagittal slices, 9 on each side of the midline. To correct for individual differences in brain size, each person's absolute cerebellar volume was divided by total intracranial volume computed from sagittal slices spanning the whole brain. Cerebellar volume was found to be significantly reduced in aged subjects compared to young ones when either absolute or normalized values were considered ($t=5.414$, $df=62$, $p<0.001$ for absolute cerebellar volume and $t=4.044$, $df=62$, $p<0.001$ for normalized volume). There were no gender differences in the extent of cerebellar atrophy with age. Since cerebellar integrity is crucial for eyeblink conditioning, this age-dependent volume loss may explain the behavioral deficit.

Supported by NIA grants PO1 AG9466 and P30 AG10161.

668.4

GENDER DIFFERENCES IN THE VULNERABILITY OF THE HIPPOCAMPAL FORMATION DURING AGING. Leyla deToledo-Morrell*, M.P. Sullivan, F. Morrell, C. Spanovic and S. Spencer. Depts. of Neurological Sciences, Psychology and Diagnostic Radiology, Rush Medical College, Chicago, IL. 60612

The hippocampal formation (HF), which is critically involved in the acquisition of certain types of new information, is especially vulnerable to the aging process. Until recently, it has been difficult to directly document such vulnerability in humans and to relate anatomical alterations to behavioral deficits. With the advent of high resolution, quantitative, magnetic resonance imaging (MRI) protocols, it has been possible to visualize detailed brain anatomy *in vivo* and to quantify age or disease induced alterations in regions of interest. Using this technology, we now report that there are ***gender*** differences in age induced changes in hippocampal anatomy. 37 healthy, aged individuals (15 males and 22 females; mean age=70, range=61-84) and 27 young subjects 13 males and 14 females; mean age=27, range=22-34) were studied with a high resolution MRI protocol, as well as the verbal and spatial versions of the Buschke "Enhanced Cued Recall" test (Buschke & Grober, *Dev. Neuropsychol.*, 1986, 19:287-307). A 3-D reconstruction program was used to compute hippocampal volume from coronal slices taken perpendicular to the long axis of the hippocampus. Left and right hippocampal volumes were derived separately. Individual differences in brain size were corrected for by dividing each person's hippocampal volume by total intracranial volume computed from sagittal slices spanning the entire brain. The right HF, but nor the left, was significantly smaller in aged males compared to their young counterparts ($F=23.62$, $p<0.001$ for the age x hemisphere interaction). This difference did not hold for females. Furthermore, aged males had a deficit in spatial, but not verbal memory. Aged females did not show any memory deficits. The differential vulnerability of the right HF in aged males fits well with the nature of the behavioral results.

Supported by NIA grants PO1 AG9466 and P30 AG10161.

668.5

EFFECTS OF AGE ON EVENT-RELATED POTENTIALS EVOKED BY WORD REPETITION IN DIRECT AND INDIRECT MEMORY TESTS. R.E. Mark, M.D. Rugg*, R.C. Roberts and J. Gilchrist. Wellcome Brain Research Group, Sch. of Psychology, Univ. of St Andrews, UK, and Dept. of Medicine, Univ. of Dundee, UK.

Event-related potentials (ERPs) were recorded to visually presented words from 13 scalp sites in samples of young (age range 19-29) and elderly (range 62-74) subjects while they performed two tasks. In the indirect task, subjects monitored a series of words in order to detect and respond to target items (animal names), which occurred on 16% of trials. 19% of non-targets repeated after a single intervening item (lag 1 repeats), and 19% repeated after a mean lag of 10 trials (range 8-12). The same trial structure was employed in the direct task, but now subjects responded to every item, discriminating between words presented for the first and the second time.

Compared to ERPs elicited by unrepeated words, words repeated in the indirect task after either lag were significantly more positive-going in both groups of subjects. This ERP repetition effect was of comparable magnitude in the two groups, but onset approximately 50 msec earlier in the young subjects. The size of the effect did not differ significantly with lag in the young group, whereas the effect of lag was highly reliable in the elderly subjects, who showed smaller effects for lag 10 repeats. In the direct task, repeated words once again elicited a positive-going shift in the ERPs of the young subjects. In contrast to the indirect task, this effect was reliably smaller for the words repeated at the longer lag. In the elderly subjects, lag 1 repeats in the direct task elicited a significant effect, which was smaller than the lag 1 effect in the young subjects. Lag 10 repeats in the direct task failed to elicit a repetition effect in the elderly subjects.

The findings show that the effects of word repetition on ERPs reflect age-related differences in brain activity associated with memory for words. In keeping with previous behavioral work, these differences are more in evidence when memory is tested directly than when it is tested indirectly.

668.6

Aging-related deficit in implicit perceptuomotor learning.
E. C. McNay* & D. B. Willingham, Neuroscience Program, University of Virginia, Charlottesville, VA 22903.

A widely-held view of the basis of age-related deficits in learning and memory has been that deficits are caused by a reduction in the level of resources available for working memory, self-initiated processing, or as part of some other model. This has been offered as an explanation for the observed reduction in performance with aging on explicit tasks and spared performance on implicit tasks.

We investigated the effect on aging on both explicit and implicit measures of perceptuomotor learning. Subjects were tested using a task which required them to trace a line presented on a computer screen using a pen held out of their sight. Baseline measurements were taken, followed by two experimental phases. In the training phase, the feedback on the screen of the subjects' movements was transformed (a constant 90 degree rotation). Subjects were told that there was a transformation, but not of the identity of the transformation. After each block of training trials, both transformation and feedback were removed for a block of test trials in which subjects were asked to directly trace the line presented. The primary measures of performance were deviation from target line (training phase, explicit measure) and signed deviation from target line (test phase, implicit measure of space recalibration).

Contrary to expectations, aged subjects showed impaired performance (after correction for age-related changes in drawing ability) in both phases, with greater deviation from the target line in the training phase and lower recalibration of their perceptuomotor space in the test phase.

668.7

SPATIAL MEMORY AS MEASURED BY A HUMAN MAZE IN AGED SUBJECTS SHOWING VARIOUS PATTERNS OF CORTISOL SECRETION AND MEMORY FUNCTION.
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Studies have shown that hypersecretion of corticosteroids result in hippocampal dysfunction and spatial memory deficit in the aged rat. We have recently shown in a group of elderly subjects that those who present a significant increase of cortisol levels over a period of 3 to 6 years present an amnesic profile as revealed by a poor declarative memory performance while aged subjects showing a moderate increase or a decrease of cortisol levels with years perform normally on these tasks. We have tested spatial memory performance in this aged population using a human maze. Three types of encoding of spatial information were measured; a spatial encoding for which the subject was shown a path by following the experimentaor through the maze and was then required to do it on his/her own; a verbal encoding for which the subject was given verbal instructions to follow in order to find his/her way in the maze; a contextual encoding for which the subject had to create a cognitive map of the maze in order to perform the path. Correlational analyses were performed between the cortisol slope of subjects as measured over a period of 3 to 6 years and their performance on the three types of encoding. The results showed significant positive correlations between the cortisol slope and the time taken to do the path in the spatial (r=0.75) and verbal encoding (r=0.45) conditions, with no significant correlation between the cortisol slopes and the contextual encoding condition (r=0.08). Since this last encoding condition did not involve any memory processing while the spatial and verbal encoding did, these results suggest that the amnesic profile described in aged subjects showing a significant increase of cortisol levels with years applies to both spatial and non-spatial information. This further confirm the animal data literature which reports a significant relationship between prolonged exposure to corticosteroids in later life and spatial memory dysfunction.

668.8

A LONGITUDINAL STUDY OF DEHYDROEPIANDROSTERONE SULFATE (DHEA-S) LEVELS, CORTISOL LEVELS AND COGNITIVE FUNCTION IN ELDERLY HUMAN SUBJECTS. S. Sharma*, A. Turken, G. Schwartz, N.P.V. Nair, M. De Leon, M.J. Meaney, R.L. Hauger and S. Lupien.
Dpt Psychiatry, VA Medical Center, UCSD, 9500 Gilman Drive, La Jolla, Ca, 92093-0603&Douglas Hospital Research Centre, McGill University, Montreal, Canada, 6875 Bld. Lasalle, Verdun, Québec, H4H-1R3

It has been postulated that dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) are markers of successful aging and life expectancy while cortisol levels may be discriminators of pathological brain aging. We have previously shown in a longitudinal study of elderly volunteers that the slope of the increase in cortisol secretion over years predicts the nature and the magnitude of the cognitive decline. We have retrospectively analyzed DHEA-S levels in this population of aged individuals for the years 1990-91, 1991-92 and 1992-93 in order to determine if DHEA-S secretion is negatively associated with cortisol hypersecretion and cognitive deficits. Subjects were retested in year 1992-93 for 24-hour cortisol secretion as well as memory function and we replicated the cortisol/memory impairment relationship previously reported. The results of the analysis of DHEA-S levels revealed decreasing DHEA-S levels in all subjects. Consistent with the hypothesis that DHEA-S may have a protective effect on the aging system, we have shown that subjects showing a significant increase in cortisol levels with years and memory impairment had the greatest decrease in DHEA-S levels. However, the reverse pattern was not observed in individuals showing decreasing cortisol levels and highly efficient cognitive function. These individuals consistently presented lower DHEA-S levels than the other subjects. Finally, although cortisol levels were significantly correlated with cognitive function, DHEA-S levels were not significantly correlated with cognitive efficiency. These results suggest that cortisol secretion during aging may be a better biological marker of cognitive decline than DHEA-S secretion.

668.9

DEMENTIA AND RESPONSE TO PAIN IN THE ELDERLY. F Porter, M Smith, JP Miller, J Morris*. Washington Univ School of Medicine, St Louis, MO 63110

To study relations among dementia, pain response and perception, heart rate and facial expression were continuously recorded during undisturbed BASEline (13±3min), PREParation (tourniquet/cleansing/needle visualized) (1±1min), VENIpuncture (2± .3min), and undisturbed RECovery (10±3min) from 44 (24 female) adults (mean age=73± 9 years) during a standard unanesthetized venipuncture. Clinical dementia ratings (0=normal, 3=severe dementia) were 14=0, 8=0.5, 12=1, 5=2, and 5=3. General anxiety and pain sensitivity were rated by subjects on 10-point visual analog scales prior to study; anticipatory and experienced anxiety and pain were rated just before or after VENI. Ratings were correlated with HR responses and dementia. HR increased from BASE to PREP and decreased from PREP to VENI (p's<.001), returning to BASE levels during VENI. A lower magnitude HR response to PREP and VENI was associated with dementia but not age or gender. Higher general pain sensitivity and higher anticipatory anxiety were positively correlated with the magnitude of HR response to VENI and negatively correlated with dementia but <40% of demented subjects were able to provide ratings. Demented subjects exhibited more facial movements to PREP (p=.06) and VENI (p=.02) than normals. More facial movements during BASE were associated with less HR increase to PREP. We conclude that the elderly exhibit a larger HR increase to stress(PREP) than pain (VENI) and that dementia may 1) decrease HR response to stress and pain; 2) minimize the magnitude of anxiety and pain ratings but self-report was limited among the demented; and 3) increase facial expression to pain. Thus, there is an apparent discordance among behavioral, physiologic and self-reported reactions to stress and pain among the demented. (NIA P50 AGO5681)

668.10

EFFECTS OF NORMAL AGING ON THE SPATIAL DISTRIBUTION OF VISUOSPATIAL ATTENTION IN VISUAL SEARCH. P.M. Greenwood*, R. Parasuraman & G.E. Alexander. Cognitive Science Lab., Catholic Univ., Washington, DC and Lab. of Neurosciences, NIA, Bethesda, MD.

Healthy elderly experience "pop-out" in visual search as readily as do young subjects when the target is easily discriminable from distracters. However, search for a poorly discriminable target, believed to require the movement of visuospatial attention from one display element to the other, proceeds more slowly in elderly compared to young subjects (Plude & Doussard-Roosevelt, 1989; Greenwood et al. 1992). Does this arise from a different spatial distribution of visuospatial attention in aging? Young subjects and two groups of elderly (aged 65-74 and 75-85) screened to exclude memory loss and dementia searched a display of 15 letters for a target defined by a conjunction of color and letter (a pink "R") and surrounded by 3,5,7 or 9 target color (pink) items, the remaining distracters being blue and green. In this type of search, subjects confine their attention to the target color items. Although RT slowed in all groups as the number of target color distracters increased, the increase was greatest in the two elderly groups. More eccentric target locations heightened the slowing of RT with increased numbers of target color distracters. This effect of eccentricity in slowing RT was greater in the elderly than in the young. However, when the target was centered in the display the young were unaffected by the number of target color distracters while the elderly were additionally slowed with each increase in target color distracters. These results suggest the spatial distribution of visuospatial attention is more diffuse in the elderly than in the young. A broader focus of visuospatial attention may contribute to age-related (1) slowing in search speed and (2) increased vulnerability of search to the presence of distracters.

668.11

SPATIAL LEARNING AND MEMORY IN FREELY MOVING MONKEYS. P.R. Rapp*1, M.T. Kansky, C.T. Stanko, and J.A. Roberts. 1Cntr. for Behavioral. Neurosci., SUNY Stony Brook, Stony Brook, NY 11794-2575, Behav. and Neurobiol. Unit, Calif. Regional Primate Res. Cntr., UC Davis, Davis, CA 95616-8542.

Tests of spatial learning and memory are among the most sensitive tasks for characterizing the effects of hippocampal dysfunction in the rat, and the present studies were intended to develop comparable procedures for monkeys. Six normal rhesus macaques (4-6 years) were tested on an octagonal platform maze (10 ft. diameter) that contained 8 hidden reward locations distributed evenly around the perimeter. A tether system confined animals to the surface of the maze, but exploration was otherwise unrestricted. Spatial working memory procedures required subjects to visit each reward location once per trial. With the exception of one monkey that failed to learn, acquisition was rapid, and subjects averaged 19.8 (±8.0) trials to achieve a criterion of 87.5% correct. Forgetting rates for spatial information were examined by imposing a delay between the 4 initial choices on each trial and the opportunity to make 4 additional choices. Accuracy declined by an average of only 12.8% (±4.3) across retention intervals ranging from 5 minutes to 4 hours, and performance remained significantly above chance at the longest delay ($p<0.005$). Rearranging the abundant extramaze cues during a 30 minute retention interval substantially impaired accuracy, implying that performance is supported by memory for the spatial relationships between these stimuli. Finally, ongoing studies of cognitive aging revealed significant learning deficits in at least a subgroup of aged rhesus monkeys. In combination, these findings are consistent with the expectation that tests of spatial learning and memory, adapted from research in rats, can provide valuable procedures for exploring hippocampal memory function in nonhuman primates. Supported by NIH grant AG10606, the CRPRC Base Grant RR00169, and a HFSPO award.

668.12

COGNITIVE CORRELATES OF TEMPORAL LOBE VOLUME AND CALLOSAL ATROPHY IN HEALTHY ELDERLY AND INCIPIENT DEMENTIA J.A. Kaye, J.S. Janowsky* and D. Howieson, Dept. of Neurology, Oregon Health Sciences Univ., VA Med. Ctr, Portland, OR. 97201

We examined the size of the corpus callosum (CC), temporal cortex, parahippocampal gyrus, and hippocampus using quantitative MRI in 26 healthy elderly (HE) "oldest-old" subjects (mean age 87). We examined the relationship between structure size and cognition. 12 of the subjects showed cognitive decline (e.g. CDR=.5-1) in the subsequent four years of follow up (Incipient Dementia, ID). Therefore, we examined structure size in HE and ID subjects when ID subjects were cognitively normal. The hippocampus was smaller in the ID subjects ($p< .05$). Temporal cortex correlated with CC size ($R=.44$, $p=.03$). The middle and posterior sectors of the CC were correlated with temporal cortex in the ID ($R's> .55$, $p's< .05$) but not the HE subjects. Significant relationships were found between memory and the hippocampal volume, and midsector of the CC over all subjects ($R's> .45$, $p's< .03$). These data suggest that atrophy precedes clinical signs of dementia. We are currently examining MRI and cognitive assessment data during subsequent years of cognitive decline in these subjects. [Supported by Dept. Veterans Affairs, NIH AG08017, NIH AG12611]

668.13

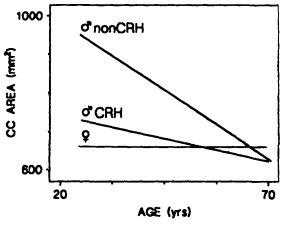
SHIFTING ATTENTION BETWEEN SENSORY MODALITIES IN HEALTHY AGING. A. Berardi*, J.V. Haxby, Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

Age-related changes in attention shifting were investigated by measuring reaction times to visual and auditory stimuli in three task conditions: a visual baseline task in which visual stimuli (rectangles) were presented to the right or left of fixation, an auditory baseline task in which auditory stimuli (tones) were presented to the right or left ear, and a shifting task in which visual and auditory stimuli were presented on alternating trials. Twenty-one young (mean age ± SD = 29.5 ± 5; range: 20-39), 20 middle-aged (mean age ± SD = 51.2 ± 6.6; range: 40-59), and 20 old subjects (mean age ± SD = 67.1 ± 4.4; range: 60-73), matched on sex and education, participated in this study. All subjects were right-handed and screened for optimal health. Reaction times were slower on the shifting task than on the baseline tasks for both auditory and visual stimuli ($p < 0.0005$, in both cases). Significant condition X group interactions revealed that slowing due to shifting was greater for both middle-aged and older subjects as compared to young subjects ($p < 0.05$, for both visual and auditory interactions). Whereas young subjects slowed 4 and 34 msec on the shifting task for visual and auditory stimuli, respectively, middle aged subjects slowed 87 and 46 msec and old subjects slowed 114 and 58 msec relative to baseline ($p < 0.05$ for all comparisons between young subjects and other groups). When reaction times on the shifting task were expressed as percent increases relative to the baseline tasks, the main group effects remained significant, indicating that age-related slowing on the shifting task was greater than would be predicted by generalized slowing. These results demonstrate that age-related inefficiency in shifting attention between sensory modalities is evident in middle aged as well as in old subjects and is not attributable to generalized slowing.

668.14

SEX AND HANDEDNESS DIFFERENCES IN CHANGE OF SIZE OF THE CORPUS CALLOSUM DURING NORMAL AGING. A. Unsal, S.F. Witelson, D.L. Kigar, M. Steiner*, Dept. of Psychiatry, McMaster Univ., Hamilton, ON, L8N 3Z5.

Advancing age is associated with a small decline in brain size. We previously reported that midsagittal area of the corpus callosum (CC) decreases sharply with age, but in men only (Witelson, NEJM, 1991). The aim of the present study was to assess, in a larger group of postmortem brain specimens from subjects varying in age from 25 to 70 yr, what the quantitative model may be between CC size, age and hand preference in men and women. A sample of 46 women and 32 men were studied, all documented to be of normal neurologic and cognitive status. Mean CC areas for men and women were 688 and 662 mm^2, respectively. For men, CC area is greater in non consistent-right-handed (nonCRH) than CRH men (725 mm^2 vs 656 mm^2, $p = .02$). For each group of men, there was a negative relationship with age [nonCRH ($r = -0.69$, $p < .01$) and CRH ($r = -0.46$, $p = .05$)]. The slopes of the regresssion lines differed significantly, indicating that the decline in CC area per year is greater in nonCRH than CRH men (R^2 change, $p < .05$). For women, no relationship was observed ($r = 0.02$): this was true whether linear or quadratic models were considered. The best predictor of CC area in women was the group mean. By age 70, all three groups approached a similar CC size, a point of callosal equality. The results suggest that CC size may have theoretical implications as well as diagnostic utility (using in vivo imaging) for cortical changes that occur with normal aging; that some sex hormonal factors influence brain aging; and in men, hemispheric lateralization affects brain change with age. Funded in part by grants NS18954 and MRC (CA) MA-10610.



668.15

THE EFFECTS OF ODORANT AND NUMBER OF TRIALS ON THE OLFACTORY EVENT-RELATED POTENTIAL IN YOUNG AND OLDER ADULTS. C.D. Morgan, J.W. Covington, C. Quiñonez, D. Ellison, D. Wester, D.L. Kalinoski*, J. Polich, C. Murphy(UCSD Medical Center, Naval Medical Center, The Scripps Research Institute, San Diego State University, San Diego, CA 92120, Monell Chemical Senses Center, Phila. PA 19104)

Olfactory event-related potentials (OERP) are currently used to differentiate groups of individuals based on peak amplitude and latency and show considerable promise as a diagnostic tool in clinical settings. In an effort toward determining clinical utility in an individual participant, the present study was designed to find the optimal parameters for recording the most robust OERPs in both young and older adults. Using three different stimulus odors (amyl acetate, geraniol, phenylethyl alcohol) the clearest and most consistent OERP waveforms were produced by amyl acetate. The average of 20 stimulus trials recorded from each participant produced a robust waveform, however, a distinct wave is also present in the average of as few as 10 trials; fewer trials result in shorter administration time, a factor which is desirable in clinical settings. Furthermore, peak amplitudes and peak latencies were found to differentiate young and older adults such that the amplitudes were smaller and latencies were longer for the older participants, indicating that age is an important factor in OERP responses. These findings will help guide future research and clinical application in olfactory assessment.
Supported by NIH grant #DC02064 to C. M.

668.16

PRE-MOVEMENT AND AUDITORY EVENT-RELATED POTENTIALS IN HEALTHY AGED SUBJECTS. M. Roe*, W. J. Evans, H. J. Michalewski and A. Starr. Departments of Psychobiology and Neurology, University of California, Irvine, CA 92717.

Pre- and post-stimulus event-related potentials were studied to examine the effects of "normal" human aging. A healthy "Old" group (mean = 80 yrs.) and "Young" group (mean = 38 yrs.) of subjects were tested. The "Old" group did not show the usual memory changes associated with age, suggesting that these subjects represent a healthy, normal aged population. Brain potentials were recorded at scalp sites Fz, Cz, Pz, C3', and C4' during an auditory target detection task and during self-paced movements. To targets, measures of accuracy, reaction time (RT), a pre-stimulus negative potential (readiness potential [RP]) and post-stimulus event-related potentials (P50, N100, P200, N200, P300) were analyzed. To self-paced movements, the average amplitude of a self-initiated RP was analyzed. No significant differences were found for accuracy or RTs between the two age groups. No significant differences for age group were found for the self-initiated RP. In contrast, pre-stimulus RP amplitudes decreased significantly with age. The post-stimulus P300 latency was significantly delayed (80 msec) and P300 amplitude was reduced (50%) for the "Old" group as compared to the "Young" group. In spite of normal performance measures, the "Old" group showed age-related changes in both latency and amplitude of several event-related components, indicating a dissociation of behavioral performance and cerebral processing of auditory signals with aging.

669.1

CORTICAL SYNAPTOGENESIS IN HEMIMEGALENCEPHALY. J.R. O'Kusky[1]* and H.V. Vinters[2]. Dept. of Pathology and Laboratory Medicine, [1]University of British Columbia, Vancouver, B.C., Canada V5Z 1M9 and [2]UCLA Medical Center, Los Angeles, CA 90024.

Histological specimens for electron microscopy were prepared from cortical resections, performed for the treatment of intractable childhood epilepsy, in 4 cases of hemimegalencephaly (HME) and 4 control cases (Rasmussen's encephalitis) ranging in age from 1 to 7 years. Stereological analyses were used to determine postnatal changes in the numerical density (N_V, contacts per mm^3) of asymmetric and symmetric synapses in the cerebral cortex. Samples included frontal, parietal and temporal cortices. In control cases the N_V of asymmetric synapses decreased after 1 year of age due to a loss of axospinous contacts with no change in the density of axodendritic or axosomatic contacts. The N_V of symmetric synapses increased gradually up to 7 years of age. In HME cases the N_V of asymmetric and symmetric synapses did not differ significantly from controls. Given the increased cortical thickness in HME, the number of synapses in a column of cerebral cortex beneath 1 mm^2 of pial surface was substantially greater than in controls. (Supported by the B.C. Health Research Foundation)

669.2

SACCADIC VARIABLES IN ADULTS WITH CEREBRAL PALSY. M.LeGare*, S.Gu, H.Zhang and S.Lee. Biomedical Engineering Program, California State University, Sacramento, CA 95819-6019.

Aimed movements require visual localization. The slow and inaccurate aimed movements in cerebral palsy (CP) may be partly due to the saccadic system which provides visuomotor support for localization. The right and left eye positions of 6 CP and 4 normal (N) adults were measured by the 7000S (Micromeasurements, Inc., Farmington, CT). Square-wave stimuli at three frequencies (0.3,0.5,0.7Hz), three amplitudes (±4,±6,±8°), horizontal and vertical dimensions were used for 18, 10s tests in a J-saccade paradigm. The right and left eye positions were pooled for this analysis of five saccadic variables. The CP group had greater than normal means for: saccadic latency (M_{CP}=0.367±0.207s; M_N=0.268±0.088s), RMS error (M_{CP}=3.588±2.693°; M_N=0.268±0.088), and fixation (M_{CP}=0.299±0.09s; M_N=0.266±0.053s). The CP group made more low velocity saccades than the N group but the acceleration profiles were comparable indicating similar organization. Therefore, the system should also exhibit motor learning. Such training may improve aimed movement in CP. Supported by: NSF-BCS9107276, CSUS-RA to ML.

669.3

DUPLICATION OF THE EYE AND CEREBRAL HEMISPHERE IN A HUMAN. T. A. Woolsey*, E. J. Stelnicki, J. L. Marsh, B.C.P. Lee, and M. J. Noetzel. Divisions of Experimental Neurology & Neurological Surgery, Plastic & Reconstructive Surgery, Neuroradiology, and Pediatric Neurology, Washington University School of Medicine, St. Louis, MO 63110.

We describe a unique case of complex craniofacial and CNS anomalies. The focus here is on aspects that relate to the nervous system. The child's skull was asymmetric (plagiocephalic) with the axis displaced to the right. However, her most overt and distinguishing feature was that the left orbit contained two eyes while the right orbit contained one normal appearing and functioning eye. Accordingly the anomaly was termed TRIOPIA. Computer assisted medical imaging was used to define intra- and extracranial structures *in vivo*. There were two left cerebral hemispheres: the anterior was gyrencephalic; the posterior lissencephalic. Other abnormalities included duplication of extraocular muscles excepting the lateral lateral rectus on the left; two left ophthalmic arteries; two left middle cerebral arteries; agenesis of the corpus callosum; duplication of the falx cerebri, sagittal dural venous sinuses and confluens of sinuses. The circle of Willis was incomplete. The brain stem was nearly symmetrical with slight reduction in the left cerebellar hemisphere and an attenuated left medullary pyramid. We propose that the embryological basis for this unprecedented anomaly was duplication, in the neural plate, of primordia for the eye and secondary prosencephalon.

Supported by NIH Grant NS 17763, the McDonnell Center for Studies of Higher Brain Function and an award from the Spastic Paralysis Foundation of the Illinois-Eastern Iowa District of the Kiwanis International.

669.4

Murine Platelet Activating Factor Acetylhydrolase: Expression Pattern and Enzymatic Activity in the Developing Brain Correlate with the Timing of Neuronal Migration

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Miller-Dieker lissencephaly, a human brain malformation associated with a hemizygous deletion in the *LIS 1* gene, is a defect in cell migration leading to an abnormal cerebral cortex. Bovine platelet-activating factor acetylhydrolase (PAF-AH) has been purified previously. PAF-AH deacylates and thereby inactivates platelet activating factor, an alkyl-ether phospholipid implicated in various aspects of neuronal development and in neuronal function. Purification studies suggest that PAF-AH consists of three subunits (29kD, 30kD and 45kD) and the 45kD subunit is identical with the coding sequence of the *LIS 1* gene. In order to study the spatial and temporal expression of the PAF-AH subunits, we cloned the cDNAs for the mouse 29kD and 30kD subunits. Both subunits are highly conserved between mouse, bovine and human. We find that the mRNAs encoding the the 29kD, 30kD and 45kD subunits are coexpressed in the cerebral cortex and in the hippocampus. Coexpression is also observed in the migrating granule cells of the developing cerebellum. PAF-AH activity measurements in cytosolic extracts show that in the cerebellum activity is highest between postnatal day 5 and 16, the time-span of granule cell migration. Correlation between elevated enzymatic activity and high levels of expression in the developing cerebellum substantiate the notion that 29kD, 30kD and 45kD subunits are associated in vivo.

669.5

SPECIFIC CORRECTION OF "ANTI-BRAIN" AUTOIMMUNITY. E.F.Bakunenko, B.B.Gnedenko, A.B.Poletaev*. Chernobyl-Test Ctr., 10 Rimskogo-Korsakova St., 127577 Moscow, Russia.

As it was found earlier, most of children, which have been born from mothers with elevated serum anti-S100 immunoreactivity, have revealed a different forms of the brain malfunctioning (mental retardations, cerebral palsy, seizure syndroms). Ability to abnormal production of anti-S100 antibodies (AB) has epygenetically transferred from mother (not from father) to offsprings and could be related to aggravation of the brain dysfunctions. It was found that maternal "tunning" of the offspring immune system based on a combination of AB-dependent modulation of specific lymphocyte repertoires, and direct transfer, and persistention of maternal "memory" lymphocytes into fetus. In experiments (rats were preliminary immunized to S100) it was found that parenteral administration of antiidiotypic AB could inhibit specific serum' anti-S100 reactivity. Also there were found two immunomodulatoric S100 fragments. The first one had at least equal efficient compared with antiidiotypic AB (selective stimulator of specific T-suppressors?). The second one significantly increased anti-S100 immunity (probably due to stimulation of a specific T-helpers). These findings may explain some aspects of pathologic anti-S100 autoimmunity as well as to be used for elaboration of a prophilactic measures.

669.6

INDUCED MICROGYRIA AND ITS EFFECTS ON CELL SIZE, CELL NUMBER, AND CELL PACKING DENSITY IN THE MEDIAL GENICULATE NUCLEUS. A.E. Herman, R.H. Fitch, A.M.Galaburda*, and G.D. Rosen. Beth Israel Hospital and Harvard Medical School, Boston, MA 02215 and CMBN, Rutgers Univ., Newark, NJ 07102.

Microgyria can be induced in otherwise normal rat neocortex by neonatal freezing injury (Humphreys et al., *J. Neuropath. Exp. Neurol.* **50**:145,1991). Adult animals with microgyria located in frontal and somatosensory cortices show deficits in fast auditory temporal processing (Fitch et al., *Cereb. Cortex* **4**:260,1994). It is not understood why damage to cortical areas far from auditory input would disrupt auditory discrimination. We have hypothesized that the developmental cortical injury results in changes in connectionally related areas, and that some of these changes involve areas important for auditory processing.

To test this hypothesis we measured cross sectional cell areas, cell packing densities, and total cell numbers in the medial geniculate nuclei (MGN) of rats who had received neonatal cortical freezing or sham injury and who had been behaviorally tested for auditory temporal processing in adulthood. Measures of cross-sectional neuronal area and cell packing density of ventral, dorsal, and medial regions of the MGN were derived using a variation of the dissector method (Williams and Rakic, *J. Comp. Neurol.* **278**:344,1988), and MGN volumes were computed based on estimates from serial sections.

Preliminary analysis has shown changes in the patterns of asymmetry in cell size between microgyric and control rats — control rats have larger neurons in the left hemisphere while microgyric animals show no asymmetry. Continued analysis will concentrate on gender differences, differences based on lesion location, and the relationship of the anatomic measures to auditory temporal processing

This work was supported, in part, by grant HD20806.

669.7

NEOCORTICAL DYSPLASIA, MICROGYRIA, AND PORENCEPHALY: COMMON ETIOLOGIES? G.D. Rosen* and A.M. Galaburda. Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

Injury to the developing cortical plate before the end of neuronal migration is generally believed to be the underlying cause of neocortical migration disorders such as molecular layer ectopias, microgyria, and porencephaly (Sarnat, *Amer. J. Dis. Children* **141**: 969,1987). It has been hypothesized that these different forms of developmental pathology result from distinct mechanisms of injury. Alternatively, they may result from a single mechanism, but only the severity of the injury varies among them.

Molecular layer ectopias and microgyria have been induced in otherwise normal neocortex by focal damage to the developing cortical plate in rats (Rosen et al., *Dev. Brain Res.* **67**:285,1992; Humphreys et al., *J. Neuropath. Exp. Neurol.* **50**:145. 1991). In the present experiment, we placed a freezing probe on one side of the skull during the first day of life (P1) for durations ranging from 2 to 20 seconds. In some rats, the procedure was repeated a second time at a location adjacent to the first. The animals were sacrificed on either P2, P4, P7, P10, P21, or P60 and brains examined with standard and immunohistochemical stains for the presence of neuropathology.

Single injury 5 seconds or longer resulted in the formation of microgyria, with the depth of the microsulcus and the volume of the lesion increasing with longer freezing times. Two-second freezing injury rarely resulted in microgyria, and instead most often led to molecular layer ectopias and/or minor laminar dysplasia. As expected, injury at two adjacent sites led to more severe disturbances than single injury, culminating in the formation of porencephalic cysts following 20 second injury. These findings indicate that a common mechanism can underlie the formation of molecular layer ectopias, microgyria, and porencephaly.

Supported, in part, by HD20806.

669.8

NEURONAL AREA IS INCREASED IN THE VENTROBASAL COMPLEX IN AUTOIMMUNE NZB MICE WITH CORTICAL ECTOPIAS G. F. Sherman*, L.V. Stone, and A.M. Galaburda. Dyslexia Research Laboratory, Beth Israel Hospital; Harvard Medical School, Boston, MA 02215.

Neuronal cell size differs in both the LGN and MGN of dyslexics in comparison to controls (Livingstone et al., PNAS 88:7943,1991; Galaburda et al., PNAS 91:8010,1994). Neurons of the magnocellular division of the LGN and of the left MGN are smaller in area. These differences may play a role in the perceptual deficits seen in dyslexia and may be the result of changes in cortical targets produced by the presence of the neocortical ectopias in layer I seen in dyslexia (Galaburda et al., PNAS 91:8010,1994). Because thalamic cell size has been measured only in dyslexics with neocortical ectopias it was not possible to directly test this relationship. We used an animal model, the New Zealand Black mouse (NZB/BINJ), that develops similar malformations in about 40% of the cases to test the relationship of molecular ectopias to neuronal size in related thalamic nuclei. Because most ectopias in NZB mice occur in the somatosensory barrel field we measured neuronal size in the ventrobasal complex nuclei VPM and VPL.

Mice were transcardially perfused with 0.9% saline followed by 10% formalin. The brains were embedded in celloidin, cut in 30 µm coronal sections, stained with cresyl violet, and mounted. Neuronal cell size was measured blind in the right and left VPL and VPM in 8 adult NZB mice with barrel field ectopias and 11 adult NZB mice without ectopias. Two sections (150 µm apart) per brain which contained VPM and VPL were selected. Cross-sectional neuronal areas in one field per section for each nucleus on both sides were measured using a stereology-derived system (Williams and Rakic, J. Comp. Neurol. 278:344,1988). The counting grid was 85 µm high, 95 µm wide and 25 µm deep. Neurons were grouped according to area into bins and X^2 values were calculated. The distribution of neuronal size differed between mice with and without ectopias for both VPL ($p< 0.001$) and VPM ($p< 0.001$). In both nuclei there were fewer small neurons and more large neurons in mice with ectopias. We suggest that the prenatal generation of molecular layer ectopias in somatosensory cortex subsequently affects the organization of related thalamic nuclei. Supported by NIH grant HD 20806.

669.9

CONNECTIVITY OF CORTICAL ECTOPIAS IN AUTOIMMUNE MICE. A. R. Jenner*, A. M. Galaburda, and G. F. Sherman. Dyslexia Research Laboratory, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

New Zealand Black mice (NZB/BINJ) and NXSM-D/Ei mice develop minor neocortical anomalies and are used as a model for the similar pathology present in the brains of dyslexics. Approximately 40% of the NZB and 80% of NXSM-D mice prenatally develop clusters of ectopic neurons in cortical layer I. Neurofilament staining of these ectopias provided preliminary evidence that the connections of these cells were aberrant. The present study was designed to look more specifically at the cortical and subcortical connections the ectopias.

Adult mice were perfused with 2% paraformaldehyde/0.1% glutaraldehyde and the brains removed from the skull. The brains were stained with methyl-green in order to visualize the surface of the brain more clearly and viewed under the dissecting microscope for ectopias. Large ectopias appear as small bumps on the surface of the brain. Ectopias were visualized in seven brains. A small crystal of DiI which labels cell membranes by passive diffusion was then placed in the middle of the ectopia just under the pial surface. The brains were stored in fixative at 37°C for up to 4 months. The brains were cut with a vibratome into 100 µm coronal sections and viewed under using a rhodamine filter set in order to visualize the DiI.

DiI brightly labeled the ectopias and in all cases there was a distinctive bundle of labeled fibers extending from the ectopic cells through the deeper layers of the cortex. This bundle of fibers then either entered the corpus callosum or the internal capsule. Six ectopias were in the somatosensory barrel cortex and one was in the hindlimb sensorimotor cortex. Depending on the location of the ectopia within the barrel cortex labeling was seen in thalamic nuclei VPM, and/or Po. The hindlimb ectopia was connected with VPL. Cortico-cortical connections were also seen between ectopias in barrel cortex and both secondary somatosensory and primary motor cortices. These DiI studies provide the first conclusive evidence that the neurons within the ectopias are connected both to other cortical architectonic areas and thalamic nuclei. Supported by NIH grant HD 20806 & the Sackler Scholarship

669.10

ULTRASTRUCTURAL ANALYSIS OF NEOCORTICAL ECTOPIAS IN NXSM-D RECOMBINANT INBRED MICE. G.W. Boehm*, M.W. Goss, A.J. Seaman, A.M. Galaburda, V.H. Denenberg and G.F. Sherman. Biobehavioral Sciences Graduate Degree Program, Univ. of Connecticut, Storrs, CT 06269; Central EM Facility, Univ. of Connecticut Health Center, Farmington, CT 06032; and Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

Focal disruptions of the laminar organization of the cortex, similar to those found in post-mortem analyses of human dyslexic cortices, have been reported in autoimmune NZB and BXSB inbred mice (Sherman *et al.*, *Acta Neuropath.*, 74:239, 1987). These strains have since become useful animal models of this pathology. The small abnormalities produced by a breach in the pial-glial membrane have been shown to form in utero, and be associated with highly specific learning deficits (Sherman *et al.*, *Neurosci. Abs.*, 18: 1446; Denenberg *et al.*, *Brain Res.*, 562:98, 1991).

This study complements prior work done at the light microscopic level by reporting findings from transmission electron microscopic analysis of the cellular structure of neurons and glia residing in and around the apex of ectopias, in a recombinant inbred strain derived from progenitor NZB and SM mouse strains, the NXSM-D (Eicher & Lee, *Genetics*, 125: 431, 1990). Six ectopias from four NXSM-D males (2 weanlings, 2 adults) were examined. In addition to being out of position, cells inside ectopic clusters had multiple ultrastructural abnormalities. The most striking abnormality was the large, apparently ballooned dendrites noted in nearly all of the ectopias. The vacuous nature of many of these dendrites, without any similar cytoskeletal damage to neurons on either side of the ectopia, was suggestive of localized excitotoxic damage. Abnormal numbers of lysosomes frequently observed inside ectopias also argue for ongoing cellular damage. Additionally, unusual deposits of extracellular protein and extended basal laminae were seen between ectopic cell processes. Approximately half of the ectopias had what appear to be either thin extensions of the pia or rather extensive pericyte septa bordering the edges of the ectopia. Ultrastructural abnormalities appear to be more severe in adult mice. Supported in part by NIH grant H-20806.

669.11

HIPPOCAMPAL DEGENERATION IN APP-C100 MICE IS ASSOCIATED WITH EXTRAMEDULLARY HEMOPOIESIS. M. L. Oster-Granite*, J. R. Greenan, D. McPhie, and R. L. Neve. Div. Biomed. Sci., Univ. CA, Riverside, CA 92521-0121 and Depart. Genetics, Harvard Univ. Sch. Med., Boston, MA 02178.

We have previously described the pathogenesis of massive pyramidal and granule cell loss in the hippocampi of mice transgenic for an amyloid precursor protein construct (APP-C100) driven by a neuron specific dystrophin promoter. When the reporter sequence FLAG is added to the construct, we have observed extensive degeneration as early as one year of age in some these new transgenic lines. Lysosome-like inclusions, immunoreactive for the antibodies that recognize epitopes in the C-100 construct, appear in increasing numbers of cells and increasing numbers appear in individual neurons in many regions of the CNS as the animals age. Examination of cerebellum, cerebral cortices, spinal cord, hypothalamus, and striatum have revealed no significant neuronal cell loss relative to that significant neuronal destruction observed in the hippocampal formation of individual transgenic animals. In addition, many of the APP-C100 mice exhibit cardiomegaly, hepatosplenomegaly, and extramedullary hemopoiesis reminiscent of that occurring in human beings with anaplastic anemia or in individuals with Down Syndrome. The earlier and more rapid degeneration of the hippocampus in the FLAG construct is accompanied more frequently by megakaryocytic abnormalities which may be associated with the platelet abnormalities observed in individuals with DS and some individuals with AD. Supported by HD19932 (MLOG) and NS28695 (RLN).

669.12

DEVELOPMENTAL EXPRESSION OF THE AMYLOID PRECURSOR PROTEIN AND TAU IN A MOUSE MODEL OF DOWN SYNDROME. M.L. Lacey-Casem*, C.B. Kuo, and M.L. Oster-Granite. Div. of Biomedical Sciences, University of California, Riverside, CA 92521.

The amyloid precursor protein (APP) and the microtubule associated protein, tau, are both key components in the Alzheimer's-like neuropathology of Down Syndrome (DS). A correlation has recently been found between the expression of these two important proteins in DS, suggesting either that APP and tau are under similar transcriptional regulation or that the overexpression of APP influences the expression of tau. In order to study the interaction of APP and tau gene expression, we have made use of an animal model of DS, the Trisomy 16 (Ts16) mouse.

The levels of APP and tau gene expression were determined by quantitation of the respective mRNAs by the method of reverse transcription-PCR. Total RNA was isolated from normal and Ts16 fetuses from 13 to 17 days gestation and cDNAs for all APP and tau isoforms were amplified and quantitated by digital image analysis of ethidium bromide stained gels. All reactions were standardized relative to GAPDH, a constitutively expressed protein.

We have found that the expression of both APP and tau follow a similar developmental pattern in the normal mouse. This result is consistent with APP and tau being under similar transcriptional control. We have also confirmed the observation that APP expression is elevated two fold in the Ts16 fetus when compared to its normal littermate. Interestingly, the level of tau expression in the Ts16 fetus is also elevated, but only from day 15 on. This latter observation argues that increased tau expression is not due to triplication of the tau gene. The pattern of tau expression in the Ts16 mouse suggests that Ts16 neurons are more sensitive to some event or signal, occurring at 15 days gestation, which influences tau gene expression. Supported by NIH1F32NS09496.

669.13

ALZEIMER'S TYPE NEUROFIBRILLARY DEGENERATION IN BRAIN WARTS M. A. Morán* and P. Gómez-Ramos Morphology Dep., Autonomous Univ. Sch. of Med., Madrid. Spain

Verrucose dysplasias (VD) of the cerebral cortex are developmental abnormalities characterized by a nodular shape and a disrupted cytoarchitecture of upper layers. In three autopsied elderly individuals (two without neurologic disorders and one with motor neuron disease), VD presented abundant Alzheimer's type neurofibrillary degeneration (ND), which immunoreacted with 5E2 and AT8 Abs, disclosed cholinesterase activity, and were thioflavin-S positive. This ND was conspicuous in layers II and III abutting the external borders of the dysplasia, as well as in layers V, VI, and in the underlying white matter. All VD contained cell-sparse areas within them, around which a peak of ND was seen. We suggest that alterations in the neuropil around neurons disturbed in their migration make them vulnerable to undergo cytoskeletal changes. Additional micro-environmental anomalies related to hypoxia-ischemia in these lesions are proposed as contributing factors for ND, pointing to the potential of VD as a source of material to study unresolved issues such as the way ND may be related to ischemia. *Supported by F.I.S. 93/0198 Spain.*

669.14

STEARYL-NLE^{17}-VIP PROTECTS AGAINST DEVELOPMENTAL RETARDATION IN APOE-DEFICIENT MICE AND AGAINST NEURODEGENERATION IN ALZHEIMER'S DISEASE-RELATED MODELS. I. Gozes*, M. Bachar, A. Bardea, A. Reshef, S. Rubinrout, M. Fridkin and D.E.Brenneman. Dept. Clin. Biochem. Sackler Med. Sch. Tel Aviv Univ. Israel, 69978; Dept. Organic Chem. Weizmann Inst. Rehovot, Israel, 76100; Sect. Develop. and Molec. Pharmacol. LDN, NIH, Bethesda, MD, USA 20892.

Stearyl-Nle^{17}-VIP is a novel agonist of vasoactive intestinal peptide (VIP) exhibiting a 100-fold greater potency than the parent molecule and specificity for a VIP receptor associated with neuronal survival (J. Pharmacol. Exp. Therap. 273:161,1995). The new molecule contains two chemical modifications in VIP, the addition of an N-terminal long chain fatty acid and the substitution of the methionine in position 17 with norleucine (Nle). In the present study, the developmental and protective effects of stearyl-Nle^{17}-VIP were investigated *in vivo* using mice deficient in apolipoprotein E (apoE) (Cell 71: 343,1992). The ApoE-deficient mice were tested for the development of behavioral milestones and were found to be significantly retarded in their acquisition of forelimb placing behavior (postnatal day 11-13) as compared to control animals (postnatal day 2-5). A two day delay in the acquisition of cliff aversion behavior also was observed in these mice. Chronic daily injections (s.c.) of stearyl-Nle^{17}-VIP to apoE-deficient new born pups resulted in a significant improvement and acquisition of behavioral milestones, similar to the development of control animals. Similarly, in Alzheimer's-related models (e.g. beta amyloid toxicity *in vitro* and cholinotoxicity *in vivo*), stearyl-Nle^{17}-VIP protected against accelerated cortical neuron death and impairments in learning and memory. Stearyl-Nle^{17}-VIP in particular and lipophilic neuropeptides in general may provide a novel route to drug design for neurodegenerative diseases. *Dedicated to the memory of the late Prof. Shlomo Eisenberg.* Supported by: Fujimoto Pharmaceutical Corp.

669.15

ALTERED MEMBRANE RESISTANCE AND REDUCED OUTWARD CONDUCTANCE IN CULTURED TRISOMY 16 MOUSE TONGUE MUSCLE CELLS SUGGEST A MECHANISM FOR THE HYPOTONIA IN HUMAN DOWN SYNDROME S. Peng, Z. Galdzicki*, and S. I. Rapoport. Lab of Neuroscience, NIA. NIH. Bethesda, MD 20892

Trisomy 16 (Ts16) mouse is a genetic animal model of Down syndrome (DS; human Ts21). Young DS patients develop a characteristic hypotonia. It has been reported that the tongue muscle is abnormal in DS. A whole-cell patch-clamp method was used to study membrane properties of isolated Ts16 and diploid control tongue muscle cells.

Muscle cell cultures were prepared from diploid control and Ts16 mouse fetus tongues on the 16th day of gestation. Cells can be maintained for 21 or 23 days. Electrophysiological recordings were done in vitro in normal physiological solutions with 1 mM $CdCl_2$ on cells cultured between day 7 and 14. Before recording, cells became spherical after a few hours of exposure to 0.1 μM colcemid. Membrane resistance (R_m) and capacitance (C_m) were measured at a holding potential of -60 mV. Mean Ts16 cell R_m was 372 MΩ (±45; n=17), whereas R_m of control was 513 MΩ (±39; n=31). Thus the Ts16 cell R_m was 27.5% less than that of control. We did not detect significant difference in C_m between Ts16 and control cells. Furthermore, we applied 15 steps of depolarization voltage with 10 mV increments to elicit total membrane current at holding potential of -60 mV. The mean of normalized maximum outward conductance ($G_{max.out}/C_m$) was 497 pS/pF (±80; n=17) and 843 pS/pF (±83; n=31) for Ts16 and control cells respectively. Thus the $G_{max.out}/C_m$ of the Ts16 cells was 41% lower than that of control, but there was no significant difference in inward current or conductance. Therefore, the cause of hypotonia could be the increased Cl^- conductance.

BETA-AMYLOID: ApoE II

670.1

RATS TREATED WITH ANTISENSE OLIGONUCLEOTIDES TO APP AND APO-E RETAIN PLACE, CUE, AND MOTOR MEMORY. C.A. Marotta*[1], S. Agrawal[2], D.P. Binsack[3]. Dept. of Psychiatry and Human Behavior[1,3],and Dept. Neuroscience[1] Brown University, Providence R.I. 02912; and, Hybridon, Inc.[2], Worcester, MA 01605.

Antisense compounds can be applied to modulate the expression of gene products implicated in a variety of disorders, including Alzheimer's Disease (AD). Thus, we designed phosphorothioate oligonucleotides complementary to amyloid precursor protein (APP) mRNA and to apolipoprotein E (APO-E) mRNA. In a previous report we showed that anti-APP can modulate the expression of APP in PC12 cells (Cell. Molec. Neurobiol. 14:425-437; 1994). The most common symptom of AD involves the disruption of memory. Thus, any potential therapeutic agent requires initial assessment with respect to its impact on behavioral functions in normal animals. Male rats were injected (i.v.) with: 1) anti-APP; 2) anti-APO-E; 3) saline; or 4) an unrelated (nonsense) oligonucleotide at dosages of 2.5 or 5.0 mg/kg. All rats were trained in a multi-component water maze task which simultaneously assessed long-term and short-term place memory, cue memory, and motor memory. Antisense oligonucleotides produced no deleterious effects on normal rats when tested for place, cue, and motor memory at the dosages tested. Safety and efficacy assessments are continuing to evaluate the therapeutic potential of antisense compounds in the treatment of AD.

670.2

EXPRESSION OF THE LOW DENSITY LIPOPROTEIN RECEPTOR FAMILY MEMBER gp330 IN RESPONSE TO EXPERIMENTAL LESION MODELS OF ALZHEIMER DISEASE. G.M. Pasinetti[1*], M.Z. Kounnas[2], W.S. Argraves[2], and C.E. Finch[1]. [1]Division of Neurogerontology, Andrus Gerontology Center and Dept. of Biological Sciences, USC, Los Angeles, CA 90089, [2]J.H. Holland Lab., Dept. of Biochem., American Red Cross, Rockville, MD 20855

Alzheimer plaques contain the apolipoproteins apoJ (clusterin) and apoE in complexes with the amyloid β-peptide (Aβ), which could have roles in the metabolism of Aβ. The LDL-receptor family member gp330 contains a shared binding site for both apoJ and apoE (Kounnas et al., *J. Biol. Chem.* 1995, in press), whereas the LDL receptor related protein (LRP) binds apoE but not apoJ. ApoE-LRP, but also apoJ-gp330 complexes can be internalized through endocytosis and might be an important clearing mechanism of Aß from neuropil for lysosomal Aß degradation. This study surveyed gp330 and LRP, and the 39 kDa LDL receptor associated protein (RAP) for changes in response to brain lesions that model features of Alzheimer disease. During responses to hippocampal deafferentation by perforant pathway transection, gp330 immunoreactivity was induced in hippocampal neurons of the CA1-CA3 subdivision and subiculum. Similarly, after infusion of synthetic aggregated Aβ1-42 peptide, surviving hippocampal neurons showed elevated immunoreactivity for gp330. ApoJ was also found as immunoreactive deposits in the vicinity of the infused Aβ. Hippocampal deafferentation increased LRP immunoreactivity in glial-rich zones, but did not alter the regional distribution of RAP. The findings highlight the coexpression of gp330 and one of its ligands apoJ at sites of brain injury which is consistent with the possibility that gp330 may be functioning to mediate clearance of apoJ at these sites. This work was supported by the Nathan W. & Margaret T. Shock Aging Research Foundation to GMP by the NIA (AG-07909) to CEF and NIH (DK45598) to WSA.

670.3

APP751 (PROTEASE NEXIN 2):PROTEASE COMPLEXES ARE INTERNALIZED VIA THE LDL RECEPTOR-RELATED PROTEIN (LRP). M. F. Knauer*, R. A. Orlando, and C. G. Glabe. Dept. of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717.

Protease Nexin 2 (PN2), a secreted isoform of the amyloid precursor protein (APP751), inhibits the binding protein for epidermal growth factor (EGFBP) by forming PN2:protease complexes. In the present studies we have investigated the mechanism by which PN2:EGFBP complexes are cleared by human fibroblasts. Our data indicate that the internalization of the PN2:EGFBP complex by human fibroblasts is mediated by the LDL receptor-related protein (LRP), which binds multiple ligands including ApoE. Complex binding and internalization were inhibited 50% and 80%, respectively, by the 39 kDa LRP receptor associated protein (RAP), which has been shown to block the binding of all ligands to LRP. In addition when EGFBP was added to surface labeled cells overexpressing APP751 there was a significant increase in the internalization of transmembrane APP751. More importantly, this increase was blocked by the addition of RAP. Taken together, these data demonstrate that the transmembrane bound form of APP751 acts as a protease inhibitor, and that its internalization is LRP dependent. This suggests an alternative mechanism by which amyloidogenic APP751 can be re-internalized into cells after it has been targeted to the plasma membrane, and may provide a mechanistic basis for the generation of the beta peptide which is a seminal component of senile plaques in Alzheimer's disease.

670.4

BINDING OF ß-AMYLOID PEPTIDE (Aß) TO APOLIPOPROTEINS E3 AND E4. L. M. Shaffer*, N. J. Richter-Cook, R. Gupta-Bansal and K. R. Brunden, Gliatech, Inc., Cleveland, OH 44122.

Apolipoprotein E (apoE) is found associated with senile plaques in Alzheimer's disease (AD), and relatively recent data reveal that individuals expressing the apoE4 isoform have an increased probability of developing AD. Previous studies demonstrated that apoE4 and the more common apoE3 isoform both bind Aß, the major component of senile plaques. However, only SDS and heat stable complexes were quantified, and conflicting results were obtained regarding the relative affinities of the apoE isoforms for Aß. Since there is a likelihood that apoE might form SDS-labile complexes with Aß, we have utilized a solid-phase binding assay to determine the affinity of recombinant apoE3 and apoE4 for fibrillar $A\beta_{1-40}$. The apoE isoforms were immobilized in 96-well plates, and fibrillar preparations of Aß were subsequently allowed to interact with the coated wells. Bound amyloid peptide was detected with a specific monoclonal antibody, followed by addition of a peroxidase-conjugated anti-mouse antibody. Aß showed saturable binding to both apoE3 and apoE4. There were not dramatic differences in the affinity of Aß for either apoE isoform at pH 7.4, with K_d values of approximately 100-200 nM. To ensure that immobilization of apoE did not expose artifactual Aß binding sites, experiments were performed in which increasing concentrations of apoE3 and apoE4 were added to the binding solutions to compete for Aß association with immobilized apoE. A dose-dependent inhibition of Aß binding was seen with solution-phase apoE3 and apoE4, with K_i values that were in general agreement with the K_d values mentioned above. These data suggest that both apoE3 and apoE4 are capable of relatively high-affinity interactions with fibrillar Aß. This is in agreement with previous work demonstrating that apoE immunoreactivity was comparable in plaques from AD patients homozygous for the E3 and E4 isoforms.

670.5

APOLIPOPROTEIN E AGGREGATES WITH SOLUBLE AMYLOID BETA IN ALZHEIMER'S DISEASE AND NORMAL BRAINS. G. Angelini, C. Russo, D. Zaccheo[#], X. Xu[&,*], J.K. Teller[&], P. Gambetti[&] and M. Tabaton[#]. Advanced Biotechnology Center, Genova, and [#]Institute of Anatomy, University of Genova, Italy, and [&]Division of Neuropathology, Case Western Reserve University, Cleveland, OH 44106.

Apolipoprotein E (ApoE) allele 4 is a genetic risk factor in sporadic and familial Alzheimer's disease (AD). Recent studies in vitro suggest that ApoE4 may play a role in modulating amyloid beta (Aβ) polymerization through a preferential binding. We detected and analyzed ApoE - Aβ complexes in the cerebral cortex of AD and normal subjects. An anti-ApoE antiserum was used for immunoprecipitation from buffer-soluble fraction of the brain. Immunoprecipitates were separated by gel electrophoresis, blotted, and analyzed with monoclonal antibodies against ApoE and Aβ (Mab 6E10). Apart from two free peptides (~4 and 3.7 kDa) detected with 6E10, a band migrating at ~41 kDa was detected with both anti-ApoE and anti-Aβ Mabs, indicating that the band corresponded to an ApoE - Aβ complex. Surprisingly, we also found the same 41 kDa band in the extracts from control brains in which soluble Aβ was undetectable. Our findings suggest that ApoE may sequester soluble Aβ in the normal brain. Direct analysis of the ApoE - Aβ complex and comparsion of the complex in AD cases homozygous for ApoE3 and ApoE4 are in progress. Supported by NIA grants AG0812, AGNS08155, AG08992, the Britton Fund, and grants from NATO and Telethon (E126).

670.6

APOLIPOPROTEIN E POLYMORPHISM AND RECEPTOR INTERACTION IN ALZHEIMER DISEASE. H. Soininen*, O. Heinonen, S. Ylä-Herttuala, P. Riekkinen Sr. Dept. of Neurology and AIV-Institute, University and University Hospital, Kuopio, P.O.Box 1627, 70211 Kuopio, FINLAND.

Alzheimer disease (AD) is associated with an increased prevalence of apolipoprotein E (ApoE) ε4. ApoE is a plasma protein that binds to low-density lipoprotein (LDL) receptor and the α_2-macroglobulin receptor/LDL receptor-related protein (α_2MR/LRP). In the central nervous system, ApoE is produced and secreted by astrocytes. In AD, ApoE is bound to senile plaques (SPs), tangles as well as to cerebrovascular amyloid. Colocalization of ApoE and α_2MR/LRP to SPs suggests that these molecules may play a role in amyloid accumulation. The aim of this study was to investigate the ApoE and α_2MR/LRP interactions in AD patients with different ApoE genotypes. We determined immunopositivity for the anti-β-amyloid (Aβ), anti-ApoE and anti-α_2MR/LRP-α in the frontal cortex of 3 ApoE ε44, 1 ε34 and 2 ε33 AD patients and 2 aged ε33 controls. Immunostainings were done for adjacent 50 μm-thick free floating sections using an avidin-biotin-peroxidase system. The major results were: 1) All AD patients showed a high number of Aβ immunopositive plaques in the frontal cortex, 2) The number of ApoE immunopositive plaques was higher in AD ε44 patients compared to ε33 patients, 3) Immunopositivity for anti-α_2MR/LRP-α was increased in AD cases compared to controls, 4) The number of anti-α_2MR/LRP-α positive astrocytes was increased in AD ε44 patients compared to those with ε33. These results underlie the genetic association of ApoE alleles with the severity of AD pathology.

670.7

PROMOTION OF THE NEUROTOXICITY OF ALZHEIMER Aβ PROTEIN BY THE PATHOLOGICAL CHAPERONES ACT AND APOE4: INHIBITION BY Aβ-RELATED PEPTIDES AND APOE2. J. Ma*, [&]H. Bryan Brewer, and H Potter. Department of Neurobiology, Harvard Medical School, Boston, MA 02115; [&]National Heart and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

The amyloid β-protein (Aβ) is a major component deposited in the senile plaques of Alzheimer's disease brain. We and others have previously found that two amyloid associated proteins, apolipoprotein E4 (ApoE4) and α_1-antichymotrypsin (ACT), possess strong amyloid filament-promoting activity and these proteins have been proposed as pathological chaperones for the formation of amyloid (Ma and Potter, 1994, *Nature*, 373:92-94, Sanan et al., 1994, *J. Clin. Invest.* 94:860-869 and Wisniewski et. al 1994, *Am. J. Pathol.* 145:1030-1035). Here we report the effects of the amyloid filaments promoted by ACT and apoE4 on the viability of primary human cortical neurons. ACT- and apoE4-induced amyloid filaments were more neurotoxic than amyloid filaments formed in the absence of pathological chaperones, as judged by a colometric MTT assay. Preincubation of ACT and apoE4 with Aβ-related peptide, or of apoE4 with apoE2, reduced their ability to promote the formation of neurotoxic Aβ filaments. The maximal neuroprotection effect of Aβ-related peptides and apoE2 can be observed at 100 nM concentration. These data suggest that ACT and apoE4 act as pathological chaperones—promoting the generation of amyloid filaments in Alzheimer's disease, which, in turn, lead to neuronal cell death. The ability of Aβ-related peptides and apoE2 at nM concentration to serve as "antipathological chaperones" by reducing the ACT and apoE4 neurotoxic filament-promoting function, may provide the basis for a potential therapy for Alzheimer's disease.

670.8

ASTROCYTE- APOLIPOPROTEIN E (APOE) ASSOCIATIONS IN SENILE PLAQUES IN ALZHEIMER'S DISEASE (AD) AND VASCULAR LESIONS: A REGIONAL IMMUNOHISTOCHEMICAL STUDY. Y. Shao*, M. Gearing, and S. S. Mirra. Department of Pathology and Laboratory Medicine, VA Medical Center and Emory University School of Medicine, Atlanta, GA 30322.

While ApoE and ß-amyloid (Aß) colocalize in senile plaques in cortex and cerebellum in AD, the Aß-positive, predominantly diffuse plaques in the striatum do not exhibit ApoE immunoreactivity. As astrocytes are a major source of ApoE in brain, we investigated potential regional differences in the ability of astrocytes to produce ApoE that might affect Aß processing and progression of AD pathology. Using antibodies to ApoE, glial fibrillary acidic protein (GFAP) and Aß, we compared the pattern of immunoreactivity in senile plaques in AD autopsy tissue with that of reactive astrocytes surrounding subacute and old infarcts in both AD and non-AD cases. We found GFAP and ApoE immunoreactivity but no Aß label in cell bodies and processes of reactive astrocytes in vascular lesions within cerebral cortex, striatum, and cerebellum. In contrast, while many senile plaques in AD cortex evidenced prominent GFAP-positive astrocytes, ApoE immunoreactivity was not observed within these cells but instead paralleled that of Aß. Prominent ApoE label of astrocyte cytoplasm was seen, however, in neighboring zones of infarction. Our findings suggest that, in AD and non-AD brains, reactive astrocytes are capable of upregulating ApoE regardless of the location of injury. Moreover, the apparent absence of ApoE label in senile plaque astrocytes may reflect decreased ApoE expression and/or increased release related to ApoE-Aß binding or other factors. Supported by VA Merit Award and AG10130.

670.9

BIOCHEMICAL QUANTITATION OF ALZHEIMER Aß PEPTIDE AND APOLIPOPROTEIN E AGGREGATED INTO AMYLOID FIBRILS. L. Buée*,[1] B. Permanne,[1] J. Pérez-Tur,[1] J-Ch. Lambert,[1] J.Ph. David,[1,2] P. Vermersch,[1] F. Ghozali,[2] F. Pasquier,[3] F. Lebert,[3] H. Petit,[3] C. Di Menza,[2] M.C. Chartier-Harlin,[1] A. Delacourte[1]
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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the presence of amyloid deposits and neurofibrillary tangles in the cerebral cortex. The main component of amyloid deposits is a 42 amino-acids peptide referred to as Aß. AD is associated with an increased frequency of the apolipoprotein E (ApoE) ε4 allele. ApoE is a secreted glycoprotein involved in cholesterol transport and neurite outgrowth. In AD, ApoE is also found in amyloid deposits. ApoE binding to Aß peptide could lead to their aggregation into amyloid fibrils. Conversely, ApoE ε2 allele may be neuroprotective. 101 brains were obtained at autopsy from elderly patients at different stages of amyloid deposition including 61 demented and 40 non-demented cases. For all of the cases, classical neuropathological examination was performed. Aß peptide and ApoE aggregated into amyloid fibrils was also quantified by dot-blot immunoassay. A strong correlation was found between this Aß biochemical quantitation and number of amyloid deposits (per mm^2) determined by neuropathological examination. Using this method, we are able to determine the amount of Aß within amyloid deposits in the cerebral cortex of typical AD patients. These data should allow to set up a better model of Alzheimer type amyloidosis.

ApoE detection was not proportional to Aß-immunoreactivity obtained by dot-blot immunoassay. These data suggest that ApoE binding to Aß within amyloid deposits may be related to particular ApoE variants. Thus, ApoE was genotyped in 78 out of the 101 cases. Interestingly, cases having at least one ε2 allele always demonstrated lower Aß amounts compared to other subjects. These data suggest that ApoE ε2 allele may facilitate clearance of Aß peptide.

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670.10

PHYSIOLOGICAL APOLIPOPROTEIN E ε3 AND ε4 ISOFORMS PROMOTE FIBRILLOGENESIS OF Aß (1-42) IN A THIOFLAVINE T ASSAY. D. Sweeney[1], Z. Zhou[1], R. Martins[1], H. LeVine[2], J. Cheetham[3], J. Breslow[4], J. Smith[4], P. Greengard[3] and S. Gandy[1*1]Dept. of Neurology & Neuroscience, Cornell Univ. Medical College, 1300 York Ave., New York, NY 10021; [2]Dept. of Neuroscience Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48106; [3]Lab. of Molecular & Cellular Neuroscience, and [4]Lab. of Biochemical Genetics & Metabolism, The Rockefeller Univ., 1230 York Ave., New York, NY 10021

Genetic-neuropathological correlation has established that apoE ε4 specifies increased cerebral amyloid plaque density. Thus, an important starting point for understanding the role of apoE in Alzheimer disease is the elucidation of the relevant apoE isoform-dependent interactions with Aß. Here we report the application of a thioflavine T based assay of Aß fibrillogenesis kinetics to the study of apoE/Aß interactions, and use this assay to study modulation of Aß fibrillogenesis using metal ions, recombinant apoE isoforms, or physiological apoE isoforms from stably transfected CHO cells. Aluminum, iron and zinc promoted fibrillogenesis of synthetic Aß (1-40) and Aß (1-42) by 3-5 fold ($p<0.001$) following incubations of 1, 4 or 7 days. Recombinant baculoviral apoE isoforms had no obvious effect on fibrillogenesis of Aß (1-40) or Aß (1-42) under the conditions studied. Physiological apoE ε3 or ε4 isoforms, produced in conditioned serum- free media, stimulated fibrillogenesis of Aß(1-42) by approximately 1.8 fold ($p< 0.01$) as compared with conditioned media from CHO cells transfected with an irrelevant construct. In contrast, fibrillogenesis of Aß (1-40) was not stimulated by physiological apoE isoforms under the conditions studied. These results provide evidence that physiological apoE isoforms can demonstrate profibrillogenic activity. The similarity in the profibrillogenic activities of ε3 and ε4 isoforms raises the possibility that interactions other than (or in addition to) apoE-mediated Aß fibrillogenesis might contribute to the apoE ε4 isoform specificity associated with increased plaque density.

670.11

ISOFORM-SPECIFIC EFFECT OF APOLIPOPROTEIN E ON THE ASSOCIATION OF β-AMYLOID WITH NEURO-2A MOUSE NEUROBLASTOMA AND RAW264 MACROPHAGE CELLS. Z. Zhou[1,2], J. Smith[1], F. Plum*[2], J. Breslow[1], P. Greengard[1], and S. Gandy[1,2].
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Alzheimer's disease is characterized by senile plaques formed mainly from β-amyloid peptide (Aβ). Genetic analyses have revealed an allele specific association of apolipoprotein E (apoE) isoforms with the relative risk for Alzheimer's disease. In order to investigate the possible existence of an apoE isoform-dependent effect on the clearance of Aβ, synthetic Aβ(1-40) was labeled by iodination and incubated with Neuro-2a or RAW264 cells in the presence of human apoE ε3- or ε4- containing conditioned medium or control conditioned medium. After a 4 hr incubation at 37°C, cells were washed and treated with trypsin to remove surface bound Aβ. The trypsin resistant, cell-associated Aβ was quantitated by measuring the radioactivity associated with the cell pellet. When Neuro-2a cells were used together with conditioned medium prepared from stably transfected CHO cells, the cell-associated Aβ in the presence of apoE ε3 was ~30% lower than that which was cell-associated in the presence of apoE ε4 ($p < 0.001$). However, when RAW264 cells were incubated with conditioned medium prepared from transfected RAW264 cells or control conditioned medium, the cell-associated Aβ in the presence of apoE ε3 conditioned medium was ~10% higher than that which was cell-associated in the presence of apoE ε4 conditioned medium ($p < 0.05$). These results suggest that apoE can have an isoform-specific effect on the association of Aβ with cells. The different patterns of Aβ association in these two cell types suggest that cell type-dependent factors are also involved in this process.

670.12

APOLIPOPROTEIN E ALLELES IN CEREBRAL AMYLOID ANGIOPATHY AND INTRACEREBRAL HEMORRHAGE ASSOCIATED WITH ALZHEIMER'S DISEASE (AD)

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The presence of apolipoprotein E (*APOE*)- E4 allele has been implicated as a risk factor for AD. We examined the occurrence of *APOE* E4 alleles in AD associated with cerebral amyloid angiopathy (CAA) and other vascular lesions including intracerebral hemorrhage. We found significantly high frequency of the *APOE* E4 allele in AD subjects exhibiting moderate to severe CAA. This association was further corroborated in three other non-AD subjects with severe CAA in the absence of significant neocortical amyloid deposition, who were all homozygous for *APOE* E4 allele. Compared to all AD group, The frequency of the allele was also higher in AD cases with other vascular lesions such as multiple infarcts, ischemic white matter lesions, and petechial and subarachnoid hemorrhages. As previously reported, we confirm that the frequency of the *APOE* E4 allele in the diffuse Lewy body variant of AD is intermediate between controls and AD subjects. Our results suggest *APOE* E4 allele to be a significant factor in the development of CAA in AD. The possibility exists that *APOE* may be a specific factor in vascular abnormalities associated with AD.

Supported by grants from NIA and ADRDA.

670.13

APOLIPOPROTEIN E (APOE) FAILS TO ENHANCE THE EFFECT OF ß-AMYLOID ON PHOSPHOLIPASE C ACTIVITY AND ON CELLULAR CALCIUM SIGNALLING Henrike Hartmann*, Anne Eckert and Walter E. Müller
Central Institute of Mental Health, Dept. Psychopharmacology, J5, 68159 Mannheim, Germany

The major component of senile plaques in Alzheimer's disease is ß-amyloid (ßA) which has neurotoxic properties and may contribute to neurodegeneration in AD. Another risk factor for sporadic AD is the ε4 allele of ApoE. Colocalization of ßA and ApoE has been observed in senile plaques and ApoE complexes with ßA to form stable fibrillay complexes. Recent work has demonstrated an amplifying effect of various ßA fragments on neuronal Ca^{2+} signalling and on phosphoinositide (PI) hydrolysis. ApoE also enhances the rise in intracellular calcium ($[Ca^{2+}]_i$) following neuronal depolarization. The aim of the present study was to investigate if preincubation of ßA with ApoE affects neuronal PI-hydrolysis or $[Ca^{2+}]_i$ in a different way than ßA or ApoE alone. For the determination of the PI-hydrolysis mechanically dissociated mouse brain cells were loaded with ^{3}H-myo-inositol and depolarized with KCl. $[Ca^{2+}]_i$ was evaluated by the use of fura-2. ßA1-43, ApoE and ßA/ApoE (1/10) were dissolved in PBS and preincubated at 37°C for 2 days. ßA1-43 (≥ 1nmol/l) enhanced the KCl (10mmol/l) induced inositolphosphate (IP) accumulation in a concentration-dependent manner. In the presence of 1µmol/l ßA1-43 the amplification was about 30% above IP accumulation induced by KCl alone. This effect was restricted to submaximal depolarization of the cells with low KCl concentrations (≤20mmol/l). Incubation of the cells with ApoE (100nmol/l) enhanced the KCl-induced IP accumulation to about 20% above control. In contrast to the effect of ßA the ApoE-induced amplification was also seen at higher KCl concentrations, indicating that different mechanisms might be involved in the enhanced PI-hydrolysis induced by ßA and ApoE. Coincubation of ßA with ApoE did not result in further enhancement of the effect of ßA1-43 on PI-hydrolysis alone. Comparable effects were seen on the level of $[Ca^{2+}]_i$, where ßA1-43 and ApoE amplified the KCl-induced rise in $[Ca^{2+}]_i$ without further enhancement of this effect by ßA/ApoE. Therefore in our model no evidence was found that ApoE by enhancing ßA aggregation amplifies the effect of ßA on cellular signalling.

670.14

BINDING OF APOLIPOPROTEIN E2 TO ß-AMYLOID: COMPARISON TO APOLIPOPROTEIN E3 AND E4. T.M. Pederson, D.E. Frail*, R.E. Metzger, A.M. Manelli, C.A. Reardon, M.T. Falduto, G.S. Getz and M.J. LaDu. Neuroscience Discovery, Abbott Laboratories, Abbott Park, IL 60064 and Dept. of Pathology, University of Chicago, Chicago, IL 60637.

In humans, apolipoprotein E (apoE) has three major isoforms, E2 (Cys^{112}, Cys^{158}), E3 (Cys^{112}, Arg^{158}), and E4 (Arg^{112}, Arg^{158}). ApoE4 is a genetic risk factor for Alzheimer's disease (AD). Recent evidence suggests that apoE2 may protect against late-onset AD. We previously demonstrated that native preparations of apoE3 from conditioned media or human plasma bind to Aß with 20-fold greater avidity than apoE4. This preferential binding of Aß to apoE3 was abolished with a purification process which includes delipidation and denaturation. Here we expand these observations to include Aß binding to native apoE2, the isoform deficient in binding to the low density lipoprotein receptor. Human apoE2 cDNA was transiently and stably transfected into HEK-293 cells and the conditioned media was incubated with Aß1-40 peptide. Complex formation was analyzed by non-reducing, SDS-PAGE, followed by immunoblotting with either Aß or apoE antibodies. Under these conditions, apoE2 migrates as dimer, apoE3 as monomer (~70%) and dimer (~30%), and apoE4 as monomer. The presence of cysteine in apoE2 and apoE3 allow these isoforms to form disulfide-linked dimers. Initial results indicated that the apoE2 dimer-Aß complex was less abundant than either apoE3 monomer-Aß or apoE4-Aß complexes. We are currently purifying apoE2 to determine its Aß binding capacity relative to the native protein. These data underscore the notion that the more prevalent apoE3 isoform may provide protection from AD by binding to Aß, while apoE2 and apoE4 may lack this function.

670.15

EFFECT OF APOLIPOPROTEIN E ISOFORMS ON ß-AMYLOID INDUCED TOXICITY IN RAT PRIMARY HIPPOCAMPAL CULTURES. M. T. Falduto*, M.J. LaDu, A.M. Manelli, G.S. Getz, and P.S. Puttfarcken. Dept. of Neuroscience, Abbott Laboratories, Abbott Park, IL 60064 and Dept. of Pathology, University of Chicago, Chicago, IL 60637.

Apolipoprotein (apoE), particularly the e4 allele, is genetically linked to Alzheimer's disease. Immunostaining colocalizes apoE with ß-amyloid (Aß) to senile plaques. In vitro, Aß has been shown to be neurotoxic. While the role of apoE in the pathogenesis of the AD is unknown, one possibility is that it protects against Aß-induced neurotoxicity. As the hippocampus is the region of the brain most affected by AD, we evaluated the effect of both native and purified preparations of apoE3 and apoE4 on Aß-induced toxicity in primary cultures of rat hippocampal neurons. Morphological and biochemical changes were assessed following plating with apoE (30 µg/ml) ± 15 µM Aß(1-42). We have previously observed that apoE in conditioned media from HEK cells stably transfected with human apoE3 or apoE4 cDNA is lipid-associated. Using this native preparation as the source of apoE, both apoE isoforms protected against Aß-induced neurotoxicity in primary rat hippocampal cultures. Toxicity was assessed by neurite length and cell viability (MTT assay) measurements made 3 days after exposure to the peptide. These results correlated with microscopic examination. Using purified apoE, again both isoforms protected against Aß-induced toxicity. The addition of exogenous ß-migrating very low density lipoprotein (40 µg cholesterol/ml) alone did not provide protection against Aß-induced toxicity or enhance the protection provided by purified apoE3 and apoE4 alone. These data suggest a role for apoE in protecting against Aß- induced neurotoxicity.

670.16

EFFECT OF APOLIPOPROTEIN E ISOFORMS ON THE DEVELOPMENT OF RAT PRIMARY HIPPOCAMPAL CULTURES. P.S. Puttfarcken*, A.M. Manelli, M.T. Falduto, G.S. Getz, and M.J. LaDu. Dept. of Neuroscience, Abbott Laboratories, Abbott Park, IL 60064 and Dept. of Pathology, University of Chicago, Chicago, IL 60637.

The correlation between the e4 allele of apolipoprotein E (apoE) and Alzheimer's disease is well established. However, the role of apoE in both normal and pathological brain processes remains unknown. Previous work has demonstrated that apoE has neurotrophic effects on cultured dorsal root ganglion cells, possibly related to cholesterol delivery in the developing cultures. To begin to understand the function of apoE in CNS neurons, we treated rat primary hippocampal cultures with native or purified preparations of human apoE3 or apoE4 (30 µg/ml). Morphological changes were assessed by microscopic examination and measurements of neurite length at 1 and 3 days following plating. We have previously observed that apoE in conditioned media from HEK cells stably transfected with human apoE3 or apoE4 cDNA is lipid-associated. Using this native preparation as the source of apoE, apoE3 exhibited greater neurotrophic actions than apoE4 at 1 day in culture. This effect was attenuated by day 3. A comparable result was observed using purified apoE isoforms. Neurotrophic effects were also observed 1 day following the addition of ß-migrating very low density lipoproteins (>20 µg cholesterol/ml). None of these treatments produced significant changes in cell viability. These results demonstrate that this system is suitable for investigating the role of apoE in the CNS.

670.17

EXPRESSION AND CHARACTERIZATION OF ALL THREE APOLIPOPROTEIN E ISOFORMS (E2, E3, AND E4) IN INSECT CELLS. W.J. Checovich* and T. Burke. PanVera Corporation, Madison, WI 53711.

Apolipoprotein (Apo-) E is thought to play a role in the progression of Alzheimer's Disease by way of its lipid transport role or by virtue of its interaction with either β-amyloid protein, the microtubule protein *tau*, or both. There are three primary Apo-E isoforms, E2, E3, and E4, which differ from each other in one or two critical amino acids.

We have purified all three human Apo-E isoforms after baculovirus-mediated expression in insect cells. Human recombinant Apo-E (hrApo-E) has an apparent molecular weight of 34 kDa. Two-dimensional gel electrophoresis reveals a complicated isoform pattern which is reminiscent of the human serum Apo E pattern; along with the primary hrApo-E band, three-to-four additional bands occur at more acidic isoelectric points (pI), possibly representing sialylated and deaminated forms of Apo-E. The pI's of hrApo-E2, E3, and E4 are 6.25, 6.35, and 6.7 respectively. hrApo-E, reconstituted in DMPC (dimyristylphosphatidylcholine) liposomes, competes with ^{125}I-LDL for binding to the LDL receptor. All three isoforms also bind to β-amyloid protein and *tau*.

BETA-AMYLOID: CELLULAR EFFECTS I

671.1

A PROTEIN KINASE CASCADE STIMULATED BY Aβ PEPTIDES: PKC, FAK AND FYN C. Zhang*, G. Krafft, and W.L. Klein Dept. of Neurobiol. & Physiol., Northwestern University, Evanston, IL 60208

It recently has been discovered that neuronal responses to Aβ peptides include a selective change in signal transduction associated with protein tyrosine phosphorylation (Zhang, et al, J. Biol. Chem., 1994, 269:25247). This change is most evident in FAK, an unusual protein tyrosine kinase (Clark and Brugge, 1995, Science 268:233) coupled to integrins and other receptors, to actin regulation, and to the ras signaling pathway. With respect to dose, kinetics, dependence on cell differentiation and requirement for Aβ aggregation, the evoked FAK Tyr (P) correlates with Aβ neurotoxicity, in both rat and human nerve cell lines. FAK thus provides an interesting molecular landmark to elucidate the cascade of intracellular events triggered by Aβ aggregates. We now show that aggregates of Aβ stimulate FAK-*fyn* association, indicating that the Aβ-stimulation of FAK Tyr(P) is of functional consequence, and that PKC participates upstream in the signaling pathway from Aβ to FAK Tyr(P). How the cascade is triggered is unknown, although stimulation of FAK Tyr(P) by A23187 and by mas-7 suggest that Aβ could access the cascade by promoting Ca++ leak or modifying G-protein function. While their potential down-stream consequences are extensive, the coupling of PKC, FAK and *fyn* to mitogenic pathways are especially intriguing; recent reviews (Heinz, TINS 1993, 18:157) have suggested that ectopic entry to the cell cycle in neurons could cause programmed cell death. Because *fyn* immunoreactivity is significantly increased in Alzheimer's-afflicted neurons (Shirazi, Neuroreport 1993, 4:435), this cascade could be a significant factor in Alzheimer's pathogenesis, especially if amyloid-evoked disregulation of *fyn* activity should prove to be neurodegenerative.

671.2

SECRETED FORM OF AMYLOID PRECURSOR PROTEIN PRIMES NAIVE PC12 CELLS FOR NEUROTROPHIC EFFECTS OF NERVE GROWTH FACTOR. W.C. Wallace*, W.E. Lyons, C. Akar, and V. Haroutunian. National Institute on Aging/GRC, Baltimore, MD 21224, Mount Sinai School of Medicine, New York.

Subcortical lesions of various neurotransmitter systems result in the *in vivo* induction and secretion of amyloid precursor protein (APP) in the corresponding projection fields of the cortex. In order to understand the physiological role of the secreted APP, we have studied the interaction of purified secreted human APP_{751} with NGF. APP induced a relatively modest outgrowth of neurites (approximately 25% of cells exhibited neurites) from naive PC12 cells when present at 50 to 100 ng/ml concentrations in the media. The induction was rapid (maximal effect after one day) but shortlived (decline in number of neurites after 3 days). In contrast, NGF-induced neurite outgrowth required at least 3 days and was present at maximum numbers after 7 days. Simultaneous addition of suboptimal concentrations of APP (1 ng/ml) and NGF (1-5 ng/ml) dramatically elevated neurite outgrowth, suggesting a synergistic interaction of the two factors. Similar effects were observed when APP was bound to laminin and extracellular matrix (matrigel) substrata. The ability of low levels of NGF (5 ng/ml) to induce neurites was increased 3- to 4-fold with APP present in the substrata. In addition, low concentrations of APP (which did not induce neurites) primed naive PC12 cells for subsequent neurite induction by NGF. Naive cells were treated with either 10 ng/ml APP or 50 ng/ml NGF for 4 days. The cells were triturated and replated in the presence of various concentrations of either APP or NGF. Compared to NGF primed cells, those cells primed with APP exhibited the most rapid and dramatic response to subsequent treatment by NGF, showing neurites 2 hours after exposure to NGF. APP-induced neurite outgrowth was minimal for cells initially primed with either APP or NGF. These results implicate APP as a co-growth factor in the injured cortex where it may act to make damaged neurons more responsive to the low levels of NGF.

671.3

NEURITE EXTENSION ASSAY DETECTS THE DEFECTIVE FUNCTION DUE TO SWEDISH MUTATION IN AMYLOID B/A4 PROTEIN (APP) H.L. Li,J.M. Roch, M. Pawlik*, S. Sisodia and T. Saitoh. Sch. of Med., Dept. of Neurosciences, Univ. of Ca, San Diego, La Jolla CA 92093.

Alzheimer's disease (AD) is characterized by selective loss of large neurons, decrease in presynaptic terminal density, and amyloid accumulation in the brain. The abnormal expression or processing of APP might play a critical role in these pathogenic process. To test this hypothesis, the human Swedish mutant APP695 cDNA, was stably transfected to a clonal CNS neuronal line, B103, and an African green monkey kidney cell line, COS-1, and compared with wild type APP695 tranfected cell lines. B103 cells transfected with Swedish mutant APP695 construct developed neurites significantly slower than those transfected with wild type APP695 when plated in a serum-free defined medium. Neurite outgrowth of parent B103 cells promoted by the addition of conditioned medium (CM) from wild type APP695 overproducing cells. However, neurite outgrowth was inhibited by the CM from Swedish mutant APP695 overproducing cells. The effect of Swedish mutant on neurites was not entirely due to the production of Aβ peptide. We determined that the secreted form of APP (sAPP) through α-secretase cleavage (sAPPα) could block the effect of Aβ, whereas sAPP produced by β-secretase (sAPPβ) could not under the identical condition. These results demonstrate the importance of the C-terminal cleavage site (α or β) on the trophic activity of sAPP regulating the deleterious effect of Aβ. We suggest that not only Aβ but also the secreted form of APP is critically involved in the pathogenesis of AD.

671.4

PHYSIOLOGIC LEVELS OF ß-AMYLOID (Aß) ACTIVATE PI3-KINASE AND PROMOTE NEURITE OUTGROWTH IN PC12 CELLS. Y. Luo*, T. Sunderland and B. Wolozin Section on Geriatric Psychiatry, NIMH/NIH, Bethesda, MD 20892-1264

The aß peptide plays an important role in Alzheimer's disease but is also present in blood serum and cerebrospinal fluid at 225 - 625 pM. The potential physiologic actions of low level aß have not been explored. We have found that picomolar doses of aß can stimulate tyrosine phosphorylation of neuronal cells. One of the phosphasyl proteins responding to aß is phosphatidylinositol-3 kinase (PI3 kinase). Three independent lines of evidence support the hypothesis that aß is activating PI3 kinase through a tyrosine kinase mediated mechanism. Immunoblotting studies show that aß induces tyrosine phosphorylation of p85 as well as binding of the p85 subunit of PI3 kinase to tyrosine phosphorylated proteins. Studies of glycosylated membrane proteins show that aß induces a translocation of p85 to membrane bound proteins, which are likely to be receptors. Finally, direct determination of lipid kinase products show that aß increases the activity of PI3 kinase in anti-phosphotyrosine immunoprecipitates. Activation of PI3 kinase by aß also stimulates PC12 cells to send out processes. Processes are evident within 8 hrs of application of 10 - 1000 pM aß; doses above this inhibit process outgrowth. Wortmannin, a selective inhibitor of PI3 kinase, blocks this response. Thus, physiologic levels of aß stimulate tyrosine phosphorylation which leads to the activation of PI3 kinase and stimulation of neurite outgrowth.

671.5

CONNEXIN43 (Cx43) AND GAP JUNCTIONS IN PC12 CELLS OVEREXPRESSING β/A4 AMYLOID AND Cx43 ELEVATION IN ALZHEIMER'S DISEASE. J.I. Nagy*, M.Z. Hossain, B.D. Lynn, W. Li, E.L. Hertzberg and C.A. Marotta. Dept. Physiol., Univ. of Manitoba, Winnipeg, Canada, Dept. of Neurosci., Albert Einstein College of Med., Bronx, NY and Dept. of Psych., Brown Univ., Providence, R.I.

Previous studies have shown that PC12 cells overexpressing β/A4 amyloid peptide display altered morphology characterized by extensive intercellular appositions. To determine if these features are accompanied by gap junctional communication, we examined connexin43 (Cx43) in normal and β/A4-transfected PC12 cells. Two β/A4-transfected PC12 clones exhibited induced Cx43 expression by Western blotting, intracellular and plasma membrane-associated Cx43 in some cells of cultures processed by immunofluorescence, dye-transfer between some cells microinjected with Lucifer Yellow, gap junctions between cells examined by EM, and transmission of intercellular Ca^{++} waves in cultures imaged with fura-2. Normal and vector-transfected PC12 cells exhibited none of these properties. Increased immunofluorescence in some clusters of β/A4-transfected cells was also observed with a monoclonal antibody against Cx32. Comparisons of Cx43 in normal and Alzheimer's Disease (AD) brain indicated that cortical areas with numerous β/A4 amyloid plaques contained increased levels of Cx43 and some plaques corresponded to sites of elevated Cx43 immunoreactivity. If β/A4 amyloid peptide causes aberrant gap junctional communication through induction or increased expression of connexins in cells, this may compromise cellular homeostatsis and exacerbate pathological conditions in amyloid plaques.

671.6

β-AMYLOID DECREASES ADHESION OF FIBROBLASTS IN CULTURE.

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Fibroblasts from Alzheimer's Disease (AD) victims demonstrate several abnormalities. These include reduced spreading, decreased adherence on plastic culture plates and absence of a 113-pS potassium channel. The channel defect was simulated in normal fibroblasts by the addition of β-amyloid. To study the effects of β-amyloid on the adherence of cultured mouse fibroblasts, cells were exposed to the 40 residue form under a variety of conditions. By acutely or chronically administering β-amyloid to cultured cells, we were able to demonstrate that the protein interferes with normal cell adherence on a number of substrates. Rates of adhesion with acutely added β-amyloid were decreased on the laminin derived peptide IKVAV and on collagen I but not on plastic alone. The effect was concentration dependent. β-amyloid, when used as a substrate, decreased adherence . It is apparent that β-amyloid can have deleterious effects even on non-neuronal cell types.

671.7

PHYSIOLOGIC LEVELS OF ß-AMYLOID AUGMENT PLATELET AGGREGATION. B. Wolozin*, C. Jones, R. Dukoff, N. R. Shulman and T. Sunderland. Section on Geriatric Psychiatry, NIMH and Clinical Hemotology Branch, NIDDK, Bethesda, MD 20892.

ß-amyloid is constitutively secreted by many cells and is normally present in our blood and CSF at levels ranging from 225-625 pM. Although micromolar levels of aggregated aß are toxic to neurons, the functions of soluble aß is unknown. We now report that aß appears to play an important physiologic role in augmenting platelet aggregation. Addition of 1 nM $aß_{1-40}$ to gel filtered platelets in Tyrodes buffer increased the sensitivity of the platelets to ADP-induced aggregation approximately 2-fold. Addition of aß alone, however, did not induce platelet aggregation, both ADP and fibrinogen were required, and RGDS, which blocks fibrinogen-integrin binding, prevented aggregation. The aß peptide augmented ADP-induced aggregation at doses of aß beginning at 100 pM, peaking at 1 nM aß and evident up to 1 μM. The reverse $aß_{40-1}$ sequence and the $aß_{25-35}$ sequence were both inactive, while $aß_{1-16}$ showed weak (25%) enhancement of platelet aggregation. Addition of wortmannin, which inhibits PI3 kinase, reduced the effects of aß on aggregation, suggesting that PI3 kinase is involved in the aß-response. Biochemical studies show that aß induces rapid increases in tyrosine phosphorylation of a protein at 180 kDa, which occurs even in the absence of ADP or fibrinogen, suggesting that this process is directly related to binding of aß. These results show that ß-amyloid at physiological plasma levels markedly potentiates platelet aggregation possibly by activating a specific tyrosine kinase-linked cellular receptor.

671.8

UBIQUITIN-DEPENDENT PROTEIN DEGRADATION IS INHIBITED BY AMYLOID BETA-PROTEIN. L. Gregori*, M. Pereira#, and D. Goldgaber. Dept. of Psychiatry and Behavioral Science, Sch. of Medicine, SUNY at Stony Brook, Stony Brook, NY 11794.# Dept. of Pharmacology, Mt. Sinai Medical Center, New York, NY 10029.

Ubiquitin and ubiquitin conjugate immunoreactivity is typically observed in neurofibrillary tangles and inclusion bodies in the brains of individual affected by neurodegenerative disorders such as Alzheimer's disease and Down's syndrome. In addition to ubiquitin, amyloid β-protein (Aβ) immunostaining is also detected in neurofibrillary tangles. We investigated the correlation between the presence of Aβ and the accumulation of ubiquitin conjugates. In our *in vitro* studies using radiolabeled lysozyme as the substrate, we found that synthetic $Aβ_{1-40}$ inhibited ubiquitin-dependent degradation pathway. In the presence of Aβ, lysozyme-ubiquitin conjugates were normally formed and subjected to deubiquitination. However, their degradation was inhibited which is consistent with Aβ affecting the multi-subunit 26S proteasome activity. We tested the catalytic subunits of the 20S proteasome and found that $Aβ_{1-40}$ selectively inhibited the chymotrypsin-like activity within the proteolytic complex. Inhibition of ubiquitin conjugate degradation by Aβ would explain the accumulation of high levels of ubiquitin conjugates which are found in neurofibrillary tangles and inclusion bodies. Furthermore, these studies identify Aβ as an inhibitor of the 26S proteasome which, if occurs *in vivo*, has important physiological significance and consequences.

671.9

EXOGENOUS AMYLOID Aß1-42 STIMULATES THE INTRACELLULAR ACCUMULATION OF NEWLY SYNTHESIZED, 4 kDa AMYLOID PEPTIDE IN THE INSOLUBLE FRACTION OF TRANSFECTED CELLS.

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Our earlier reports have indicated that intracellular Aβ1-42 aggregates alter the turn-over of APP to cause the accumulation of insoluble APP and amyloidogenic fragments. To determine whether this accumulation ultimately gives rise to the production of more Aß, we examined the insoluble fraction of Aß1-42-treated cells for the presence of 4 kDa amyloid peptides. ^{35}S-*met* labeled 4 kDa Aβ peptides were immunoprecipitated from the detergent insoluble fraction of the cell lysate by anti-Aβ antibodies. The amyloid peptides were purified by size gel filtration and reverse phase column chromatography. Sequence analysis of purified peptide indicated that some of the newly synthesized peptides are "ragged" as previously observed for the exogenously added peptide. These peptides were eluted as a broad peak by a reverse-phase HPLC; this chromatographic properties is very similar to the Aβ1-42. In addition, the purified ^{35}S-met labeled amyloid peptides form SDS-stable high molecular weight aggregates with synthetic Aß1-42, suggesting that at least a fraction of these peptides extends to residue 42. Therefore, our results suggest the intracellular Aβ1-42 aggregates serve as a nucleus for the further accumulation of additional newly synthesized Aβ peptides. Supported by NIH AG00538 and NS31230.

671.10

PERSISTENT ACTIVATION OF MICROGLIA BY LPS INFUSED WITH Aß 1-40 PEPTIDE AND PERLECAN

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Prior studies injecting LPS into rat brain to activate microglia found the inflammatory reaction resolves within two weeks of injection (Andersson et al, 1992). We have investigated the activation of microglial cells in the brain by infusion of LPS into the hippocampus in combination with the Aß 1-40 peptide and the heparan sulfate proteoglycan, perlecan. Fisher 344 rats were cannulated unilaterally in the hippocampus and infused with 200 µl of solution containing 15µg LPS, 250 µg Aß 1-40, and 70 µg perlecan in PBS delivered by an osmotic minipump at a rate of 0.5µl/h for 14 days. At eight weeks following surgery, animals were sacrificed and their brains sectioned and stained with Nissl (ribonucleoprotein particles) or Thioflavin S (amyloid), or immunostained with antibodies against GFAP (astrocytes) or the MHC II marker OX-6 (microglia).

We report a persistent activation of microglial cells following an infusion of LPS, Aß 1-40, and perlecan into the hippocampus. Eight weeks post surgery, brains contained a significantly elevated number of OX-6 immunopositive microglia in rats receiving LPS plus amyloid compared to amyloid alone ($p < 0.05$). The ipsilateral microglial response involved multiple brain regions whereas the contralateral response was confined to the hippocampus. In addition, the area of necrosis produced by the injection was significantly greater in those rats retaining amyloid deposits at the eight week time point. No effects of LPS or residual amyloid on GFAP immunoreactivity were observed. These data suggest that sustained infusion of LPS plus amyloid into the brain results in the persistent activation of microglial cells. Once activated, microglia may phagocytise deposited amyloid concomitantly initiating the neurodegeneration associated with Alzheimer's disease. Supported by the Alzheimer's Association 11RG 93-083 (DGM).

671.11

INTRAHIPPOCAMPAL AMYLOID BETA-PEPTIDE 1-42 INTERFERES TWO WEEKS LATER WITH ONE-TRIAL REWARD LEARNING.

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Amyloid beta-peptide 1-42 (Aß) is a major plaque constituent found in Alzheimers Disease (AD). Nine Sprague-Dawley rats received 7 hippocampal microinjections per side of Aß 1-42 (Abbott Labs, 9 nmol per site) in DMSO, while 7 received equivalent injections of Aß 1-42 with a scrambled amino acid sequence in DMSO, and 7 received DMSO alone. After 14 days, all rats were trained on a one-trial discriminative reward learning task using a starburst maze. Retention of this task has previously proved to be sensitive to the effects of aging, cholinergic activity and other Aß fragments. On the retention trial one day later, the Aß 1-42 group had significantly more errors than DMSO controls (Dunnett's Test). The scrambled Aß 1-42 group did not differ significantly from controls. The Aß 1-42 group also had significantly slower speed (reciprocal of latency to reward) than DMSO controls. Again, the scrambled Aß group did not significantly differ from controls. There were no significant differences on the initial training trial. The results suggest that Aß peptides may play a causal role in the memory deficits of AD.

671.12

LEVELS OF ß-AMYLOID AND ß-AMYLOID PRECURSOR PROTEIN IN HUMAN CSF ARE LINKED TO CHOLINESTERASE ACTIVITY.

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We have evaluated 110 human cerebrospinal fluid samples from individuals ranging in age from newborn to 82 years to determine their cholinesterase (ChE) activity, APP and ß/A4 levels. When examined, no age-related change in the average level of either APP or ß-amyloid was observed. However, a significant correlation was observed between ChE and APP content ($p>0.001$) and between ChE and ß-amyloid content ($p<0.001$). Upon closer examination, this correlation was found to be developmentally regulated. In neonates (0-1 month), ChE activity correlated with APP levels ($p<0.001$) but not with ß-amyloid levels. However, in the 1 month to 1 year age group, ChE activity correlated with ß-amyloid content ($p<0.001$) and not with APP. After 1 year of age, a significant correlation was found between ChE activity and APP or ß-amyloid content. This correlation also increased in significance with increasing age. A significant correlation between APP and ß-amyloid ($p<0.001$) was also found in CSF. Again, this relationship was found to be developmentally regulated and only existed in individuals over 25 years of age. The underlying mechanisms explaining the correlations presented here are not clear at this time, however, these data do suggest a link between cholinergic activity and APP processing.

671.13

CHOLINESTERASE ACTIVITIES AND LEVELS OF APP, sAß, AND COMPLEMENT PROTEINS C1q, C3 AND C9 IN CSF FROM NORMAL AND AD PATIENTS.

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It has been suggested that certain proteins in the cerebrospinal fluid (CSF) might change as a result of Alzheimer's Disease (AD). We measured several proteins and soluble amyloid peptide (sAß) in CSF samples from non-AD and AD patients. Samples of CSF from AD (21) and non-AD (15) patients had comparable levels of AChE, BuChE, APP, sAß, C1q and C9. Only, the complement protein C3 showed a statistically significant, but modest (40%) increase in AD versus non-AD CSF. A good correaltion existed between the APP and sAß measured in the AD ($R = 0.75$) and in the non-AD ($R = 0.70$) samples. AChE and sAß, and AChE and APP levels in the AD and non-AD samples were also correlated. In the AD samples, sAß and AChE, and APP and AChE had R values of 0.52 and 0.83, respectively. In non-AD samples, the R values for sAß and AChE (0.30) and APP and AChE (0.67) were less than in the AD samples. There was little correlation between the sAß and BuChE levels ($R = 0.19$) and APP and BuChE ($R = 0.18$) in the AD CSF. A better correlation between sAß and BuChE levels ($R = 0.56$) and APP and BuChE ($R = 0.44$) was seen in the non-AD CSF samples. In conclusion, we found little difference in the levels of different proteins and sAß in the CSF from AD and non-AD patients. We observed a good correlation between the levels of sAß, APP and AChE in the AD and non-AD CSF samples. However, there was a poor correlation for sAß and APP with BuChE n the AD samples, but not in the non-AD samples. This may imply that AD causes a disco-ordinated expression of certain proteins in the CSF relative to BuChE, but no major changes in the absolute protein levels.

671.14

CHRONIC BETA-AMYLOID TREATMENT: EFFECTS ON INCORPORATION OF FATTY ACIDS INTO RAT BRAIN.

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The present study evaluated the effects of chronic βA4 (1-40; Bachem, Torrance CA) administration on fatty acid incorporation into rat brain. βA4 was chronically infused intraventricularly via an osmotic mini-pump (Model 2ML1; Alza, Palo Alto CA) for 7-10 days at a concentration of 460 µM. After the infusion, fatty acid metabolism was evaluated using an *in vivo* method developed in this laboratory (Robinson et al., Brain Res. Reviews 17:187, 1992). Three radiolabeled fatty acids including [1-^{14}C]AA (Amersham, Arlington Heights IL; 170 µCi/kg), [1-^{14}C]DHA (Dupont NEN, Boston MA; 110 µCi/kg) and [9,10-^{3}H]PAM (Dupont NEN; 6.7 mCi/kg), reflecting structural and functional cellular roles, were infused i.v. in awake animals. Biochemical and histological analyses showed no effect due to the presence of βA4. However, *in vitro* tests, using the same lot (#445) of βA4, showed that it caused significant cell death in PC-12 cells. Furthermore, βA4 that had been retrieved from the pumps after the *in vivo* infusion was also toxic to PC-12 cells. A finer-grained analysis, using autoradiographic imaging, is underway. In addition, the fate of the βA4 in the rat brain is being examined using Western blotting.

671.15

BETA-AMYLOID INHIBITS PROTEOLYSIS OF TAU PROTEINS BY RABBIT RETICULOCYTE LYSATE Ronald S. Black*, Cornell Univ. Med. Coll. at Burke Medical Research Inst., White Plains, NY 10605

Neurodegeneration in Alzheimer's disease is accompanied by the accumulation of insoluble aggregates of tau proteins conjugated to ubiquitin. The DEAE-cellulose binding fraction of rabbit reticulocytes, containing enzymes of the ubiquitin degradation system (Fraction II) was used to conjugate bovine ubiquitin to tau proteins prepared from twice-cycled bovine brain microtubules. In the presence of ATP, an ATP regenerating system, and hemin, which inhibits the breakdown of ubiquitin conjugates, high molecular weight ubiquitin-conjugated tau proteins accumulated. Tau-ubiquitin conjugation did not occur in the absence of ATP. In the absence of hemin, tau-ubiquitin conjugates did not accumulate and the tau proteins were degraded in an ATP- and ubiquitin-dependent fashion. Some tau proteolysis occurred in the absence of ATP; quantitative studies using ^{125}I-labelled tau demonstrated that ATP-dependent proteolysis was 2-3 fold greater than ATP-independent proteolysis in this system. β-amyloid peptides inhibited the ATP-dependent degradation of 125-I labelled tau in the reticulocyte lysate system. Aβ(1-28) was a more potent inhibitor than was Aβ(1-40). The effect of Aβ(1-28) was dose dependent and was half maximal at 5μM peptide concentration. The degree of inhibition (40-70%) was comparable to that of 20 μM hemin. This system may provide an in-vitro model of metabolic processes relevant to the pathogenesis of AD. Supported by the NIA (AG00504)

671.16

β-AMYLOID 25-35 (βA) INDUCED TAU-IMMUNOREACTIVITY FOLLOWING INJECTIONS INTO THE AMYGDALA OF RATS *IN VIVO*. E. M. Sigurdsson*, M. J. Hejna, D. J. Magnuson, J. M. Lee and S. A. Lorens. Depts. Pharmacology and Pathology, Loyola Univ. Chicago Medical Center, Maywood IL 60153.

Alzheimer's disease (AD) is characterized histopathologically by plaques and tangles in several brain regions. To determine if βA induces tau-immunoreactivity (IR) *in vivo*, we injected βA (5.0 nmole) into the right amygdala (AMY) of rats. Control rats received vehicle (VEH) infusions. In the first experiment, βA increased tau-2-IR ipsilaterally in the AMY (βA = 226 ± 95 cells; VEH = 10 ± 7, $p < 0.001$), cingulate cortex (βA = 94 ± 30; VEH = 0, $p = 0.004$) and hippocampus (HIP) (βA = 251 ± 78; VEH = 5 ± 4, $p < 0.001$) of rats sacrificed 8 days postoperatively (PO). In the second experiment, tau-2-IR was increased in the AMY (βA = 721 ± 194; VEH = 48 ± 24, $p = 0.033$) and HIP (βA = 938 ± 84; VEH = 310 ± 161, $p = 0.013$) of rats sacrificed 32 days PO. As with tau-2 the intensity of Alz-50-IR in βA rats was greater at 32 days than 8 days. In the third experiment, immunoblots were performed on the AMY of rats sacrificed 8 days PO. Some were perfused with phosphatase inhibitors (PI), others were decapitated without perfusion. Preliminary results revealed Alz-50-IR bands of similar size in PI-perfused βA and VEH rats. Alz-50-IR bands were virtually absent in non-perfused VEH rats and normal human brain but were observed in non-perfused βA rats and AD brain. Surprisingly, no difference was seen in the density of the bands in left vs right AMY, although βA was only injected into the right AMY. No difference was observed in tau-1-IR between βA and VEH rats. These data suggest that *in vivo* βA time dependently increases tau-IR in the vicinity of the injection site as well as at distant sites, possibly by acting on nerve terminals to cause cytoskeletal alterations in axons and perikarya. Immunoblotting supports the immunohistochemical findings that βA affects the state of phosphorylation of tau proteins, suggesting an association between plaque and tangle formation in AD. The tau-1 blots indicate that βA does not induce changes in the amount of normal tau or in the proportions of its respective isoforms.

671.17

AMYLOID ß PEPTIDE (25-35) INDUCES TAU PHOSPHORYLATION AND DECREASES MICROTUBULE-FORMING ABILITY IN RAT HIPPOCAMPAL CULTURE. A. Takashima*, K. Ishiguro, K. Noguchi, G. Michel, M. Hoshi, K. Sato, M. Takahashi, T. Hoshino, T. Uchida, and K. Imahori. Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194, Japan

According to the amyloid hypothesis for the pathogenesis of AD, amyloid ß peptide (Aß) directly affects neurons, leading to neurodegeneration and tau phosphorylation, followed by the production of paired helical filament (PHF) in neurofibrillary tangles (NFT). Consistent with this idea, primary cultures of embryonic rat hippocampal neurons undergo progressive degeneration as well as expression of an epitope for phosphorylated tau after exposure to Aß. However, it is unclear whether the tau is phosphorylated at the same sites as PHF-tau, and whether the phosphorylated tau causes destabilization of microtubules in hippocampal culture after treatment with Aß. These are crucial points for understanding the relationship between Aß and phosphorylation of tau in the pathogenesis of AD. To address these points, we analyzed tau phosphorylation in a rat hippocampal culture treated with Aß (25-35). By using antibodies that recognize phosphorylation sites of tau in a phosphorylation-dependent manner, we showed that the tau phosphorylation was enhanced in at least 5 sites. The phosphorylated tau accumulated in the cytoplasmic soluble fraction and showed reduced ability to support microtubule formation, as has been observed with PHF-tau. Thus, Aß exposure could modify tau to a PHF-like state in rat hippocampal culture, suggesting that this culture system may be a useful model for studying the pathogenesis of AD.

BETA-AMYLOID: CELLULAR EFFECTS II

672.1

MODIFICATION OF MACROPHAGE INFLAMMATORY RESPONSE BY ALZHEIMER β AMYLOID PEPTIDE.

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Brain resident macrophages (microglia) have been found to be closely associated with β amyloid containing plaques in brain tissue affected by Alzheimer's disease. Immunohistochemical data suggest that these cells are activated, thus indicating that inflammatory mechanisms may be involved in the pathogenesis of Alzheimer's disease. We previously reported that activation of rat peritoneal macrophages by β amyloid peptide and its subfragment (25-35) can be readily detected in several assay systems (Klegeris et al. (1994) BBRC, 199, p.984) including respiratory burst induction.

Here we show by cytochrome c reduction that both β amyloid peptide (1-40) and its subfragment (25-35) can induce production of superoxide anion in macrophages. In addition to this short-term (minutes) response a long-term effect can be demonstrated. When macrophages are pretreated with β amyloid peptides for 1.5 hours, and subsequently stimulated with opsonized zymosan or phorbol myristate acetate, the resulting superoxide generation rate is increased significantly compared with cells not so pretreated.

It is well known that such potent macrophage activators as lipopolysaccharide, interferon γ and tumor necrosis factor α can induce (prime) macrophages into a state of enhanced respiratory burst potential. The ability of β amyloid peptides also to increase the responsiveness of macrophages to various stimulating agents, thus enhancing the cytotoxic potential of these cells, could be an important factor in the pathogenesis of slowly progressing neurodegenerative disorders, including Alzheimer's disease.

Supported by a grant from the Jack Brown and Family AD research fund.

672.2

Aβ IMPAIRS ION-MOTIVE ATPase ACTIVITIES: EVIDENCE FOR A ROLE IN LOSS OF NEURONAL Ca^{2+} HOMEOSTASIS AND CELL DEATH. R. J. Mark*[1], K. Hensley[2], D.A. Butterfield[2] and M.P. Mattson[1] [1]Dept. of Anatomy & Neurobiology and Sanders-Brown Center on Aging and [2]Dept. of Chemistry and Center of Membrane Sci, Univ of Kentucky, Lexington, KY 40536.

The amyloid β-peptide (Aβ) that accumulates as insoluble plaques in the brain in Alzheimer's disease (AD) can be directly neurotoxic and can increase neuronal vulnerability to excitotoxic insults. Exposure of cultured rat hippocampal neurons to Aβ1-40 or Aβ25-35 causes a selective reduction in Na^+/K^+-ATPase activity which precedes loss of calcium homeostasis and cell degeneration. Aβ did not impair Na^+/Ca^{2+} exchange. Experiments with ouabain, a specific inhibitor of the Na^+/K^+-ATPase, demonstrated that impairment of this enzyme was sufficient to induce an elevation of $[Ca^{2+}]_i$ and neuronal injury. Impairment of Na^+/K^+-ATPase activity was causally involved in the elevation of $[Ca^{2+}]_i$ and neurotoxicity since suppression of Na^+ influx significantly reduced Aβ- and ouabain-induced $[Ca^{2+}]_i$ elevation and neuronal death. Antioxidants prevented Aβ-induced impairment of Na^+/K^+-ATPase activity, elevation of $[Ca^{2+}]_i$ and neurotoxicity, suggesting a role for free radicals. Impairment of Na^+/K^+-ATPase appears to be mediated through an intracellular pathway, because treatment of partially purified membranes with Aβ failed to impair the pump. Some neurotrophic factors have been shown to protect neurons against Aβ toxicity. We report that bFGF attenuates Aβ-induced peroxide accumulation, impairment of the Na^+/K^+-ATPase, and neurotoxicity. We also found that Aβ impairs both the Na^+/K^+ and plasma membrane Ca^{2+}-ATPases in human synaptosomes, suggesting that impairment of the ion-motive ATPases may play a role in the pathogenesis of AD.

672.3

MODULATION OF INTRACELLULAR ATP BY Aß(1-40) IN RAT CORTICAL NEURONAL CELLS. Z. Zhang, G. J. Drzewiecki, R. E. Rydel#, S. Wright#, P. C. May and P. A. Hyslop*. Dept. CNS Research, Eli Lilly & Co., Lilly Corporate Center, Indianapolis, IN 46285. #Athena Neurociences, 800 Gateway Blvd., So. San Francisco, CA 94080.

Maintenance of intracellular ATP is critical to neuronal survival during sublethal toxic insults induced by a wide variety of mediators generated during inflammation in the CNS in Alzheimer's disease by glial activation and amyloid ß (Aß) accumulation. We sought to determine if exposure of primary cultures of rat cortical neurones to Aß(1-40) depressed intracellular ATP as an early event in the progression of the neurotoxic insult. Intracellular ATP fell in a dose-dependent manner on exposure to Aß. Previous studies from a number of labs have shown that a component of Aß toxicity may involve oxidant generation. We measured dichlorofluorescein (DCF) and dihydroethidium (DE) fluorescence as markers of intracellular H_2O_2 and O_2-. generation respectively in response to Aß. No increase in DE was observed. DCF was significantly increased up to 24 hr. Cell viability was not significantly altered up to 24 hr, but declined after 48-72 hr. The glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity is inhibited by exogenously added H_2O_2, but was not inhibited by Aß exposure up to 24 hr. The decline in intracellular ATP is unlikely to result from inhibition of this enzyme by Aß. We are continuing to evaluate the potential role of oxidant stress on cell metabolism and/or signal transduction.

Intracellular ATP (% of control)

Aß time (hr.)	50µM Aß	25µM Aß	12.5µM Aß
6	108.9±2.1	107.2±16	95.3±6.5
24	79.35±3.3	76.8±4.8	90.0±2.7
48	10.38±2.2	11.9±3.2	61.6±4.17

	DCF Fluorescence		GAPDH (mU/well)	
Aß (µM)	6 hr.	24 hr.	6 hr.	24 hr.
0	100	100	5.1±0.58	7.5±1.1
12.5	134±9.7	125±21	4.8±0.15	8.0±0.75
25	154±11.2	160±23	5.0±0.36	7.7±0.7
50	164±6.7	178±30	5.0±0.3	7.4±1.67

672.4

ß-AMYLOID PEPTIDE EXERTS A RAPID INHIBITORY EFFECT UPON A NON-MITOCHONDRIAL NADPH-OXIDASE. J.B. Davis*, S. Parvathy, M.S.G. Clark, G.W. Roberts. Molecular Neuropathology, SmithKline Beecham, Harlow, CM19 5AW, UK.

βA4 is toxic to primary and clonal neuronal cells. The cytotoxic mechanism is unknown, but is associated with an increase in intracellular hydrogen peroxide and an early cellular response involving redox pathways that reduce MTT.

βA4 (0.01-10µM) has no effect upon cellular ATP production in rat neuroblastoma B12 or PC12 cells. Depletion of mitochondria by ethidium bromide pretreatment has no effect upon basal level of MTT reduction, nor upon its modulation by βA4. These data indicate that the redox pathway involved is not the respiratory chain, commonly thought to be the site of MTT reduction. Using respiratory chain and glycolytic inhibitors [rotenone (50nM), antimycin-A (128µM), 2-deoxyglucose (13mM) (DG), iodoacetate (2µM)] we demonstrate that the redox pathway inhibited is dependent upon reducing potential generated via glycolysis, most probably NADPH. Unlike DG, βA4 does not inhibit the generation of this reducing potential, suggesting that the direct or indirect effect of βA4 is to inhibit an oxidase itself.

We conclude that an early effect of βA4 is the inhibition of a non-mitochondrial NADPH-oxidase.

672.5

Depolarization of Mitochondrial Membrane Potential Accompanies ß-amyloid-induced compromise of cellular redox potential. E.J. Hunnicutt*, Jr., J.N. Davis and J.C. Chisholm , Institute for Dementia Research, Bayer Corp., West Haven, CT 06516

Neurotoxicity of the ubiquitous ß-amyloid peptide(s) (βA4), deposited in plaques characteristic of Alzheimer's disease, varies with experimental protocol, even in vitro. Compromise in dehydrogenase activity (redox state), which accompanies treatment with neurotoxic βA4 peptides, occurs rapidly (within 15 min) in a variety of cells, and precedes cell death in cultured rat hippocampal neurons. The redox compromise is associated with dose-dependent alterations in the pattern of cellular deposition of MTT product (insoluble formazan) in each cell. The subcellular distribution of the MTT product appeared to colocalize with a rhodamine mitochondrial dye in untreated cells. Since the βA4-induced alteration occurs in all cells, it suggests that each cell possesses a component of redox which is sensitive to these peptides. We now report that βA4 also produces a rapid depolarization of mitochondrial membrane potential, as measured with the fluorescent ratiometric mitochondrial membrane potential dye, JC-1, using confocal laser microscopy. This mitochondrial depolarization occurs in parallel with the compromise in MTT-reportable redox state. These data support an early mitochondrial involvement in a cellular response to βA4, at concentrations which are selectively toxic to neurons.

672.6

ß-AMYLOID INDUCED CHANGES IN THE RESPIRATORY BURST ACTIVITY OF CULTURED RAT MICROGLIA CELLS

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Activated microglia, often associated with neuritic amyloid plaques in Alzheimer's disease (AD) brain, are likely to contribute to the progression of the disease process, e.g., by releasing neurotoxic reactive oxygen species (ROS). The mechanisms whereby microglia cells become activated, however, are largely unknown. Therefore, the aim of the present study was to examine the effect of the ß-amyloid peptide (Aß) on the respiratory burst activity of microglia cells. Microglia, isolated from neonatal rat cortices, were cultured for 24h in the presence of synthetic Aß-peptides (Bachem, California) before the respiratory burst activity was studied. Aß-peptides, previously stored at 500µM in ddH_2O at -70°C, were diluted in serum-free culture medium and added to the cultures at a concentration of 1-50µM. Superoxide anion (O_2^-) production was assayed by the (superoxide dismutase inhibitable-) O_2^--mediated reduction of iodonitrotetrazoliumviolet whereas the release of nitric oxide (NO) was determined with the Griess reagent. In Aß1-40 and Aß1-42 pretreated cultures, neither the survival and the spontaneous release of NO and O_2^- nor the LPS-mediated induction of NO production was altered. However, pretreatment for 24h with Aß1-40 (1-10µM), but not Aß1-42, resulted in a statistically significant increase (1.5 to 2 fold) in the phorbol 12-myristate 13-acetate (PMA; 0.01µg/ml) stimulated production of O_2^-. Phase contrast microscopy revealed that particularly those microglia cells located in the close vicinity of Aß fragments were highly responsive. It is concluded that, as far as the production of O_2^- is concerned, Aß1-40 acts as a priming rather than a triggering stimulus on the respiratory burst activity of cultured rat microglia cells under our conditions.

672.7

β-AMYLOID INTERACTS WITH THE INTEGRIN RECEPTOR MAC-1 RESULTING IN NITRIC OXIDE RELEASE FROM CULTURED MICROGLIA. J. Goodwin, C. Martens, and E. Uemura*. Department of Anatomy and Neuroscience Program, Iowa State University, Ames, IA 50011.

While the β-amyloid protein of Alzheimer's disease (AD) has been well characterized biochemically, its primary biological function and mode of action in cells of interest in AD has not been determined. In previous studies, β-amyloid (β25-35 and β1-40) with IFN-γ was shown to trigger nitric oxide release from cultured microglial cells. A potential receptor for this effect is the microglial β2 integrin, Mac-1. We examined the interaction of β-amyloid (β25-35) with Mac-1 by a) performing competitive binding studies between biotinylated β25-35 and FITC-labeled anti-Mac-1 antibodies to Mac-1 using immunofluorescence flow cytometry and b) observing the effects of anti-Mac-1 monoclonal antibodies (Mabs) on the microglial release of nitric oxide. For the competitive binding studies, microglial cells isolated from the hippocampi of P2-4 rats (Holtzman) were exposed to biotinylated β25-35 (R-PE labeled), FITC-labeled anti-Mac-1 Mabs, or β25-35 followed by FITC-labeled anti-Mac-1 Mabs, and analyzed for fluoresence intensity by flow cytometry. For the nitric oxide studies, harvested microglia were plated onto 24-well culture plates (pre-treated with β25-35) in the presence or absence of anti-Mac-1 Mabs, incubated for 72 hours and analyzed for nitrite release. Flow cytometry revealed β-amyloid binding and upregulation of anti-Mac-1 Mab binding to microglial cells. Cultured microglia showed a decrease in β-amyloid/IFN-γ induced NO release when treated with anti-Mac-1 Mabs. Our study suggests an interaction between β-amyloid and the microglial Mac-1 receptor resulting in NO release. Such an interaction may have implications in the neurodegeneration seen in Alzheimer's disease.

672.8

AMYLOID PRECURSOR PROTEIN INHIBITS ASCORBATE INDUCED LIPID PEROXIDATION IN HUMAN CORTEX. A.C. Andorn* and M.A. Pappolla. Dept. of Psychiat., St. Louis Univ. Hlth. Sci. Centr., and St. Louis VAMC, St. Louis, MO (63125) and Dept. of Pathol., Univ. of Texas Hlth. Sci. Centr., Houston, TE (77030).

Amyloid precursor protein (APP) is the progenitor of β-amyloid (βAM) which accumulates in fibrillar form in neuritic plaques and walls of blood vessels in Alzheimer's disease (AD). Lipid peroxidation (LP) is a result of increased oxidative damage which may play a role in the pathogenesis of AD. The interaction between APP and LP was not known. We now report that when ascorbate (0.1 mM) is used to stimulate LP in particulate membrane fragments derived from presumptive normal human prefrontal cortex, APP completely inhibits the stimulated LP. Computer assisted analysis of the merged dose-response data (N=3) showed an IC_{50} of 5.7 x 10^{-6}M. Similar analysis of data obtained using fragments of APP, showed the following IC_{50}: βAM_{25-35} (2.4 x 10^{-5}M), βAM_{1-28} (5.6 x 10^{-5}M) and βAM_{1-40} (1.7 x 10^{-3}M). βAM that had been incubated to produce the neurotoxic equivalent and then used in the experiments generated an IC_{50} of 3.6 x 10^{-5}M. These data suggest that APP and some of its cleavage products can prevent LP at certain concentrations. The physiologic relevance of this finding remains to be determined.

672.9

FREE RADICAL INVOLVEMENT IN ß-AMYLOID TOXICITY. [1]C. Cafè*, [1]C. Torri, [1]L. Bertorelli, [2]N. Angeretti, [2]E. Lucca, [2]G. Forloni, [1]F. Marzatico.
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Previous data showed that a synthetic peptide (ß 25-35) homologous to residues 25-35 of ß-protein (Aß) plays a neurotoxic action, inducing cell death by apoptosis.

There is some evidence that oxygen radical reactions play a role in Alzheimer's disease physiopathology. Furthermore oxidative stress has been shown to be able to induce neuronal death by apoptosis. The aim of this work was to investigate whether free radical reactions may play a primary role in neurodegenerative events associated with Aß deposition.

Primary cortical neurons were exposed chronically and acutely to 50 µM ß 25-35: for the acute exposure a solution of the peptide was added to the medium after 7 days of culture and the effect was observed the following day; the chronic treatment consisted of a single exposure for 3 days or repeated treatments every 2 days up to day 7 in culture.

The following parameters were evaluated: antioxidant enzymes superoxide dismutase and glutathione peroxidase, lipid peroxidation products (TBARS) and intracellular reactive oxygen species production, using a fluorescent probe, dichlorofluorescin diacetate.

ß 25-35 treatment induced a marked increase of superoxide dismutase activity only after acute exposure, while it had no effect during chronic treatment. Glutathione peroxidase showed changes only following chronic treatment. Lipid peroxidation index was unaffected during chronic treatment, while the acute exposure of neurons to ß 25-35 showed a paradoxical protective effect, being lipid peroxidation product level significantly higher in the control neurons. ROS production, measured fluorimetrically, was markedly enhanced in both chronically and acutely exposed neurons.

In conclusion, our results suggest that oxidative stress and free radical production are somehow linked to the peptide presence, and, may be, to its neurotoxicity.

672.10

POSSIBLE MECHANISMS OF APOPTOSIS INDUCED BY EXPRESSION OF MUTANT AMYLOID PRECURSOR PROTEIN IN PC12 CELLS B. Zhao[(1)]*, S. S. Sisodia[(2)], and J. W. Kusiak[(1)]. Molecular Neurobiology Unit, GRC/NIA/NIH, Baltimore, MD 21224[(1)]; Department of Pathology, Johns Hopkins University, Baltimore, MD 21205[(2)].

Mutations found in the amyloid precursor protein (APP) gene of certain familial Alzheimer's disease (AD) pedigrees provide general mechanistic implications for the disease, suggesting a crucial role for altered APP processing in developing neuropathology of AD. Several groups reported extracellular neurotoxicity of fibrillar amyloid β-peptides (Aβ). Recent evidence indicated that apoptosis is associated with AD and oxidative stress can initiate apoptosis in neurons. We previously reported increased apoptosis in PC12 cells stably transfected with constructs harboring mutant APP genes (APP692, APP693, APP717F). The present studies further investigated possible molecular and cellular mechanism(s) of mutant APP induced apoptosis in these cell lines. By Western blot analysis we showed an increased level of large carboxyl terminal fragments (CTFs) of APP within the cells expressing mutant APPs. We did not observe an increased level of Aβ by immunoprecipitation in conditioned medium from the cells expressing mutant APPs. Treatment of untransfected parental PC12 cells with conditioned medium from mutant APP transfectants did not enhance apoptosis as measured by flow cytometric analysis. When N-acetylcysteine (NAC, 25mM), a thiol antioxidant, was added to mutant cell lines prior to, but not after, the initiation of apoptosis, DNA laddering was inhibited in APP692 transfected cells. However, NAC showed no effects on apoptosis in APP717 transfected cells. These data suggest that the potentially amyloidogenic CTFs generated from mutant APPs may have neurotoxic effects on the cells intracellularly. Oxidative stress seems to be one of the mediators leading to apoptosis induced by mutant APPs, which in turn may contribute to the neuronal loss in AD.

672.11

EVIDENCE FOR DNA DAMAGE, BUT NOT APOPTOSIS, IN ALZHEIMER'S DISEASED (AD) BRAIN. L.S. Perlmutter*, A.F. Bushnell, Y.-P. Li, S. Webster, S. Wong. Institute for Dementia Research, Bayer Corp, West Haven, CT 06516.

While controversial, recent work suggests that ßA4-mediated neurotoxicity may operate via an apoptotic pathway in cultured cells. Also, a transgenic mouse line overexpressing intraneuronal ßA4 exhibits staining of neuronal nuclei with a kit designed to label the nicked ends of DNA. The present study used one such kit (Apoptag, Oncor) to label autopsy samples from AD (n = 5) and non-AD (n = 5) brains to determine the physiological relevance of apoptosis to AD Fixed samples from hippocampus, cerebellum, and Brodmann's areas (BA) 22 and 17 were vibratome-sectioned and labeled free-floating. A subset were prepared for electron microscopy. Some sections were double-labeled with thioflavine S, or immunocytochemically for ßA4 (4G8) or PHF- tau (AT8). In addition, frozen brain samples taken from an adjacent region of BA 17 (the area with the most Apoptag staining) were examined for DNA laddering.

While many more stained nuclei were evident in all AD brain regions examined as compared to non-AD, the location of labeled cells (which were overwhelmingly neurons) did not correlate with either the pattern of AD-associated neuronal loss or with the presence of AD hallmark lesions (ß-amyloid plaques, neurofibrillary tangles). At the ultrastructural level, no clear apoptotic bodies or cells with pyknotic nuclei were observed; labeled nuclei did not differ in any apparent morphologic way from unlabeled nuclei. Finally, DNA laddering was not seen in any sample from AD brain; one non-AD sample exhibited laddering that was not correlated with Apoptag staining in the fixed section. These data indicate that the AD pathogenetic process may involve fragmented or nicked DNA, but provide no evidence thus far that apoptosis is the mechanism involved.

672.12

β AMYLOID-INDUCED NEURONAL DEATH IN MURINE CORTICAL CULTURES: ATTENUATION BY PHORBOL ESTER OR HIGH K^+. J. Koh*, M. I. Behrens, S. L. Sensi, L. M. T. Canzoniero, B. J. Gwag and D. W. Choi. Dept. of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Medicine, St. Louis, MO 63110

Aggregated β amyloid peptides induce neuronal death in cultures. Although the mechanism of this death is still unknown, proposed possibilities include i) formation of Ca^{2+} ionophores; ii) destabilization of Ca^{2+} homeostasis; and iii) generation of free radicals.

Murine cortical cell cultures exposed to 10-40 µM synthetic β amyloid (β1-42; obtained from K-biologicals) developed concentration-dependent, gradually occurring neuronal death, partly blockable by the protein synthesis inhibitor, cycloheximide. This β1-42-induced neuronal death was not associated with an increase in neuronal $[Ca^{2+}]_i$, measured at 12 and 24 hr after exposure onset. Indeed, it was potentiated by lowering extracellular Ca^{2+}, or variably by addition of glutamate antagonists (10 µM MK-801 or 50 µM CNQX), or 20 µM nimodipine. Furthermore addition of the phorbol ester PKC activator, 10 nM PMA, or 30 mM K^+, reduced β1-42-induced neuronal death. Addition of trolox, superoxide dismutase/catalase, or N-arginine, did not attenuate the β1-42 neurotoxicity.

These data are consistent with an idea that β1-42-induced neuronal death may occur at least in part via mechanisms involving intracellular Ca^{2+} insufficiency and new macromolecule synthesis, and fit with other data suggesting that this death may involve apoptosis (Loo et al., PNAS, 90:7951, 1993).

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672.13

COMPARISON OF ALTERED GENE EXPRESSION DURING NEURONAL DEATH INDUCED BY NGF WITHDRAWAL, AMYLOID ß-PROTEIN TREATMENT, OR GLUCOSE DEPRIVATION. S. Estus#*, C. van Rooyen#, M. Mattson‡, E.F. Brigham§, and R.E. Rydel§. Depts. of Physiology#, Neurobiology and Anatomy‡, Sanders-Brown Center, University of Kentucky, Lexington, KY 40536, and Athena Neuroscience, Inc.§, South San Francisco, CA 94080.

We previously identified a pattern of gene expression that accompanies the neuronal apoptosis of rat sympathetic neurons induced by NGF deprivation. This temporal cascade begins with transcription factors such as *c-jun* and *c-myb*, followed by *c-fos*, *fos B*, *NGFI-A*, and *rhl*, and then two extracellular matrix proteases regulated by c-Jun and c-Fos, i.e., *transin-1* and *collagenase*. Moreover, neutralizing antibodies against the Jun family, the Fos family, or c-Jun specifically, blocked apoptosis, indicating that these gene products are necessary for apoptosis. To examine whether this genetic cascade occurs in other *in vitro* models of neuronal death, we have examined rat cortical neurons (i) treated with the Alzheimer amyloid ß-protein (Aß) or (ii) subjected to glucose deprivation. When neurons were treated with 20 µM Aß, we observed robust inductions of *c-jun*, beginning at 12 hours, followed by *c-fos* and *fos B*, beginning at 24 hours, with death (LDH release) beginning at 48 hours. Similar genetic changes were not observed after glucose deprivation. In future experimentation, we shall confirm and extend these findings. The present results suggest that the induction of *c-jun* and *c-fos* may be specific to certain types of neuronal death.

672.14

BIOLOGICAL EFFECTS OF NON-FIBRILLOGENIC β-AMYLOID AND PRP PEPTIDES. G. Forloni*, E.Lucca, N. Angeretti, L. De Gioia, L.Diomede, O.Bugiani°, F.Tagliavini° and M.Salmona, Istituto di Ricerche Farmacologiche Mario Negri, °Istituto Neurologico Besta, Milano Italy.

Cerebral amyloid-β protein deposits is a major pathological feature of Alzheimer's disease. Also in spongiform encephalopathies (SE), altered form of prion protein (PrP) aggregates in amyloid fibrils and accumulates in the brain of affected individuals. Synthetic peptides homologous to β25-35 and to PrP 106-126 fragment induced neuronal death by apoptosis *in vitro*. Furthermore, accordingly with pathological features of SE, chronic treatment with PrP 106-126 increased the proliferation rate and GFAP expression in astrocytes β 25-35 and PrP 106-126 fragments have a β-sheet structure and exhibit self-aggregation properties. Since the neurotoxicity of these peptides has been associated with their fibrillogenic activity, we synthesized amidated homologous, β 25-35-NH2 and PrP106-126-NH2, to obtain peptides with low level of amyloidogenic activity and directly test the relationship between amyloid fibrils and neuronal death or astroglial proliferation. The β-sheet conformation and the self-aggregation activity of both amidated peptides were strongly reduced as determined by circular dichroism spectroscopy analysis and electron microscopy examination. Rat hippocampal neuronal cells were chronically exposed to both amidated peptides (25-100 µM). β25-35-NH 2 compared to β 25-35 reduced its neurotoxic activity, while the neuronal cell death induced by PrP106-126-NH2 was similar to that observed by exposure to unmodified peptide. In contrast, rat astrocytes chronically exposed to PrP106-126-NH2 (25-100 µM) did not alter their proliferation rate and GFAP expression compared to control condition was unchanged. Thus, astrogliosis induced by PrP 106-126 appear associated with self-aggregation activity while toxic effect, unlike β 25-35, was independent of the peptide amyloidogenic activity.

672.15

ANALYSIS OF TRANSCRIPTIONAL CHANGES IN RESPONSE TO ß-AMYLOID INDUCED NEUROTOXICITY. T. Sunderland*, J. W. Kusiak and B. Wolozin. Section on Geriatric Psychiatry, NIMH and Molecular Neurobiology Unit, NIA, Bethesda, MD 20892.

Recent studies have shown that specific transcripts, including Jun and Fos, are upregulated during neuronal apoptosis (Freeman, et al, Neuron 12: 343-355, 1994). We have now analyzed changes in gene expression occurring during ß-amyloid (aß)-induced cell death in order to better understand the mechanism of action of aggregated aß. Cortical neurons from E18 rats were grown in culture for 10 days, transferred to serum free medium and then treated with 10 µM aged $aß_{1-42}$ for 1-72 hrs. The RNA was harvested and transcriptional changes were analyzed by semi-quantitative PCR. Several transcripts examined, including G3PDH, NGF-IA and p53, showed no changes during the course of treatment with aß. Two transcripts, Fos and Bcl X showed rapid and persistent elevation evident 4 hrs after application of aß. The levels of Jun, Bax and cyclin D1 also increased, but over a slower time course, being evident 10 - 24 hrs after treatment with aß. The levels of Bcl2 increased from 4 - 10 hrs after application of aß, but then declined as the levels of Bax increased. Such reciprocal expression of Bcl2 and Bax has been observed in other systems as well. The patterns of gene expression induced in response to aggregated aß resemble that seen during neuronal apoptosis in response to growth factor withdrawal, and set the basis of an understanding of aß-induced cell death on a molecular level.

672.16

GENERATION OF AMYLOIDOGENIC ALZHEIMER'S AMYLOID PRECURSOR PROTEIN FRAGMENTS DURING H_2O_2 INDUCED APOPTOSIS IN HUMAN NEURONAL CELLS

L. Zhang*[1], J.W. Kusiak[2], B. Zhao[2] and G. S. Roth[1] [1]Molecular Physiology & Genetics Section, Lab. of Cellular & Molecular Biology, [2]Molecular Neurobiology Unit, Lab. of Biological Chemistry, NIA/NIH, Baltimore, MD21224

A number of groups have reported apoptotic cells in Alzheimer's brain. This suggests a role for neuronal programmed cell death (apoptosis) in the development of Alzheimer's disease (AD). To explore how apoptosis may relate to AD pathophysiology, we studied the processing of amyloid precursor protein (APP) during H_2O_2 induced apoptosis in cultured human neuroblastoma cells. We found increases in amyloidogenic fragments of 12 and 16 kDa both prior to and after apparent induction of neuronal programmed cell death which was indicated by DNA laddering. The level of full length APP was dependent on the concentration of H_2O_2 used. We conclude that a neuronal apoptotic process may generate fragments containing intact βA4 peptide which is neurotoxic. Since βA4 has been recently shown to induce apoptosis, we further hypothesize that amyloidogenic fragments may induce a cycle of apoptosis which generates additional toxic fragments and such a vicious cycle may contribute to the neuronal loss during AD development.

ALZHEIMER'S DISEASE: MECHANISMS OF DEGENERATION I

673.1

DISTRIBUTION OF IL-1α-IMMUNOREACTIVE CELLS IN CORTICAL LAYERS: IMPLICATIONS FOR β-AMYLOID PRECURSOR PROTEIN-IMMUNOREACTIVE PLAQUE FORMATION. J.G. Sheng,* R.E. Mrak, and W.S.T. Griffin. Ark. Children's Hosp. Res. Inst., Dept. of Veteran's Affairs Med. Ctr., Univ. Ark. Med. Sci., Little Rock, AR 72202.

The cytokine interleukin-1 (IL-1) is a potent neurotrophic factor that is present in elevated levels in brain of Alzheimer disease. IL-1 induces excessive expression of β-amyloid precursor protein (β-APP), suggesting that IL-1 is an important pathogenic factor in plaque evolution. We used computer image analysis to determine the number, size, and immunoreactive intensity of IL-1α immunoreactive ($IL\text{-}1\alpha^+$) microglia in temporal lobe of 9 Alzheimer and 4 control patients. The number of $IL\text{-}1\alpha^+$ microglia and the average $IL\text{-}1\alpha^+$ cell soma area in cortical layers 1 and 2 were similar in Alzheimer and control patients. In contrast, in layers 3 through 6, the number of $IL\text{-}1\alpha^+$ microglia in Alzheimer's disease was significantly elevated when compared to controls (3.5-fold in layer 3 and 2-fold in layers 4, 5, and 6), as was and the IL-1α immunoreactive content of microglia (2-fold higher in layers 3, 4, and 5; and 1.2-fold higher in layer 6; $p < 0.05$). The distribution of $\beta\text{-APP}^+$ plaques in cerebral layers correlated with that of $IL\text{-}1\alpha^+$ microglia ($R = 0.99$; $P < 0.005$), as did the total immunoreactive area of these $\beta\text{-APP}^+$ plaques ($R = 0.95$; $P < 0.05$). This topographical correlation between microglial IL-1α and neuritic β-APP overexpression supports a role for IL-1 in induction of β-APP overexpression and promotion of dystrophic neurite formation in Alzheimer's disease, and thus in the evolution of diffuse amyloid deposits into neuritic β-amyloid plaques. Supported in part by NS27414, AG10208, and AG12411.

673.2

C1q IN ALZHEIMER'S DISEASE CORRELATES TO SEVERITY OF DEMENTIA AND COLOCALIZES TO MATURE PLAQUES AND NEURONS. N.C. Berchtold, B.J. Cummings, D.C. Cribbs, R. Shankle, D. McCleary, A. Afagh, J. Ulas*, A.J. Tenner, C.C. Cotman. *Institute for Brain Aging and Dementia, University of Irvine, CA 92717-4550 USA*

Numerous studies have implicated the immune system as contributing to the neuropathology present in Alzheimer's disease (AD) (See McGear et al., 1994, Eikelenbloom et al., 1994 for reviews). In particular, activation of the complement system is suspected of constituting a significant factor in AD progression. Aß is known to bind C1q. We have previously reported that levels of C1q are decreased in cerebrospinal fluid (CSF) of Alzheimer's patients, suggesting increased binding of complement proteins in the AD brain. We have expanded on this study to include a larger sample size, across a broader range of dementia scores. Further, we have increased the number of control cases. We confirmed the previous findings and report that CSF C1q levels correlate with the degree of dementia of the AD individuals. We have also investigated the localization of this inflammatory marker in brain tissue with immunohistochemical double and triple labeling techniques. Using affinity purified anti-C1q and anti ß1-42, and counter staining for ß-structure with thioflavine-S, we find that C1q colocalizes with later stage plaques, but rarely with diffuse plaques. Furthermore, numerous neurons in AD brain stained immunopositive for C1q, while minimal labeling was observed in control brain tissue. Interestingly, most C1q positive neurons were neither AT8 positive, nor PHF-1 positive. However, extracellular PHF-1 positive NFT's were C1q positive. These results implicate a complement mediated inflammatory process as an early selective event in injured or stressed neurons, in AD.

673.3

EXPRESSION OF COMPLEMENT INHIBITORS C1 INHIBITOR AND PROTECTIN (CD59) BY HUMAN NEURONS DERIVED FROM NTERA2 TERATOCARCINOMA CELLS. D.G. Walker*, J. Xiao, E.G. McGeer and P.L. McGeer. Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, B.C. Canada, V6T1Z3.

Studies of brains from Alzheimer Disease (AD) cases have shown evidence of inflammatory changes in affected tissue, as demonstrated by the presence of reactive microglia and complement proteins of the classical pathway. Activation of the complement system in brains of AD patients may be leading to the death of healthy neurons (bystander lysis). Immunohistochemical studies from this laboratory have indicated that neurons may have the ability to express certain proteins that can inhibit different stages of the complement cascade.

To explore further the potential expression by neurons of complement cascade inhibitory molecules, pure cultures of cells with the characteristics of post-mitotic human neurons were prepared from the NTERA2 teratocarcinoma cell line. Following retinoic acid treatment, purified cells were prepared for gene expression studies by short term culture in serum-free media. Cells were stimulated with ψ interferon, interleukin 1β or phorbol myristate acetate. Using the reverse transcription-polymerase chain reaction (RT-PCR) technique, C1 inhibitor gene expression was detected in a number of independent preparations of neuronal cells. C1 inhibitor expression was low or absent in unstimulated neurons, and was greatly increased by treatment of cells with ψ interferon. Protectin gene expression was detected in these cells by RT-PCR, by immunoblot analysis and by immunocytochemistry. In contrast to C1 inhibitor, protectin expression was not increased by ψ interferon stimulation. These results indicate that human neurons can express crucial complement inhibitory proteins. Understanding how these proteins can be regulated may lead to potential therapeutic strategies for affecting the progression of neurodegenerative diseases like AD.

Supported by a grant from the Jack Brown and Family AD research fund.

673.4

INFLAMMATORY MECHANISMS OF NEURODEGENERATION. THE ROLE OF THE COMPLEMENT DERIVED ANAPHYLATOXIN C5a. G. Tocco[1]*, W. Musleh[1], and G.M. Pasinetti[2]. [1]Division of Neuroscience, Hedco Neuroscience Program, USC, Los Angeles, CA 90089; [2]Division of Neurogerontology, Andrus Gerontology Center, USC, Los Angeles, CA 90089

This study addresses the role of the proinflammatory anaphylatoxin C5a in mechanisms of hippocampal neurodegeneration. We found that the pyramidal neurons in the CA3 sub-region of the hippocampal formation in congenic mice genetically deficient in complement component C5 ($C5^-$) are more susceptible to glutamate mediated neurodegeneration, relative to C5-sufficient mice ($C5^+$). Moreover, the C5 derived anaphylatoxin C5a (human recombinant) was shown to diminish glutamate-mediated neurotoxicity in vivo. Therefore, the pro-inflammatory anaphylatoxin C5a might also be a neuroprotectant. Potential mechanisms include regulation of glutamate receptors and local control of cytokine expression that may influence hippocampal responses to lesions. We found that $C5^-$ congenic mice show selective impairment of Ca^{++} dependent regulation of glutamate AMPA receptors in CA1/CA3 pyramidal neurons during response to hippocampal lesions and found that hippocampal astrocytes cultured from $C5^-$ mice have hypersecretion of the cytokines IL-6 and TNF. These data are consistent with our findings showing C5a receptor (C5aR) expression in CA1/CA3 pyramidal neurons and astrocytes of the mouse hippocampal formation. This study may lead to a better understanding of complement-mediated pathophysiology in Alzheimer disease and other neurodegenerative diseases which involve excitatory AA pathways. Ongoing studies using a C5aR knockout mouse model will clarify the role of the pro-inflammatory anaphylatoxin C5a in brain. This work was supported by the Nathan W. & Margaret T. Shock Aging Research Foundation to GMP.

673.5

COEXISTENCE OF ALZHEIMER'S DISEASE WITH HERPES VIRUS ENCEPHALITIS. P. Staub, J. Colmer, F. Denaro and D. Freed*, Texas Tech University HSC, Lubbock, TX 79430

At present there are no treatments for Alzheimer's disease but diagnostic techniques have progressed using peripheral markers and clinical tests. Problems in treatment or diagnoses can arise if Alzheimer's disease is present with other conditions that cause dementia, such as herpes virus encephalitis. In the present report we examine both the clinical progression of dementia and the histological picture of herpes virus superimposed on Alzheimer's disease. Several important points were noted: The mental status of Alzheimer's patients should be evaluated at routine intervals. If a sudden change in mental status occurs, evaluation of other medical conditions should be performed. With the advent of PCR, viral testing may be more easily done and antiviral treatment initiated if needed. Examination of the brain of this Alzheimer's patient revealed viral infection using EM, immunocytochemistry and *in situ* hybridization. In addition, the histological picture of AD was superimposed in some of the infected areas. NS31875

673.6

MERCURY VAPOR INHALATION INHIBITS BINDING OF GTP TO TUBULIN IN RAT BRAIN: A MOLECULAR LESION PRESENT IN ALZHEIMER BRAIN. F.L. Lorscheider, M.J. Vimy, J.C. Pendergrass and B.E. Haley*. Univ. of Calgary Fac. of Med., Calgary, AB T2N 4N1 CAN and Univ. of Kentucky Col. of Pharm., Lexington, KY 40536 USA.

Hg^{2+} interacts with tubulin and disassembles microtubules that maintain neurite structure. Hg vapor (Hg^0) is also continuously released from "silver" amalgam tooth fillings and is absorbed into brain (FASEB J. 9: 504-508, 1995). In the present study rats were exposed to Hg^0 4 h/day for 0,2,7,14 and 28 d at 250 μg Hg/m^3 air, a concentration present in mouth air of some humans with many amalgam fillings. Average rat brain Hg concentrations increased significantly (11-47 fold) with duration of Hg^0 exposure. By 14 d Hg^0 exposure, photoaffinity labelling of the ß-subunit of the tubulin dimer with [α^{32}P]8N_3GTP in brain homogenates was decreased 75%, upon analysis of SDS-PAGE autoradiograms. The identical neurochemical lesion of similar magnitude is evident in most Alzheimer brain homogenates when compared to human age-matched controls. Since the rate of tubulin polymerization is dependent upon binding of GTP to tubulin dimers, we conclude that chronic inhalation of low-level Hg^0 can inhibit polymerization of tubulin essential for formation of microtubules.

(Supported by: NIH GM35766; Wallace Genetic Fndn.; IAOMT)

673.7

I "AN INHIBITOR OF THE 20S PROTEASOME INDUCES A STRESS RESPONSE IN A MOUSE NEURONAL CELL LINE, MANIFESTED BY ACCUMULATION OF UBIQUITINYLATED PROTEINS AND OF THE INDUCIBLE FORM OF HSP70." Maria E. Figueiredo-Pereira*, Sophie Kurdziel & Ronald P. Magnusson, Dept. of Pharmacology, Mount Sinai School of Medicine of CUNY, N.Y., N.Y. 10021.

Ubiquitin-protein conjugates are commonly detected in neuronal brain inclusions of patients with neurodegenerative diseases suggesting that a common molecular response involving ubiquitinylated proteins occurs in chronically degenerating neurons. The ubiquitin/ATP-dependent proteolytic system (26S proteasome) plays a major role in the removal of short lived, abnormal and denatured proteins. The catalytic core of the 26S proteasome is the multicatalytic proteinase complex (MPC) or 20S proteasome. Recently, we showed that exposure of HT4 cells (a mouse neuronal cell line) for 3 hours to 25 μM of a new potent permeable peptidyl aldehyde inhibitor of MPC [Z-IE(OtBu)AL-CHO] induced the accumulation of ubiquitinylated proteins. We now show that overnight incubations with concentrations of the same MPC-inhibitor as low as 0.25 μM also produce accumulation of ubiquitinylated proteins. The ladder-like pattern and increased intensity of the immunostaining observed in Western blots of total cells extracts probed with an antibody against ubiquitin-protein conjugates depicts an increase in the accumulation of ubiquitinylated proteins as compared with that observed after shorter (3h) incubations. Overnight incubations with the same concentrations of a calpain inhibitor (Z-LL-CHO) produced no accumulation of ubiquitin-protein conjugates. Furthermore, overnight incubations with the MPC-inhibitor but not with the calpain inhibitor, led to accumulation of the inducible form of the heat shock protein HSP70i in HT4 cells. Northern blots showed that there was a concentration dependent increase in HSP70i mRNA concomitant with the increase in protein expression detected by Western blots probed with a specific antibody for HSP70i. These results suggest that malfunction of the 26S proteasome resulting from a decrease in MPC activity, induces a stress response in the neuronal cells, accumulation of ubiquitinylated proteins, and ultimately may lead to neuronal death. This mechanism may be involved in the etiology of neurodegenerative diseases. (Supported by NIH grant NS29936).

673.8

• CULTURE OF ADULT RAT NEURONS AND THEIR AGE-DEPENDENT GLUTAMATE AND LACTATE TOXICITY. • P.J. Price[1]* and G.J. Brewer[2]. [1]GIBCO/Life Technologies, Grand Island, NY 14072 and [2]Southern Illinois University School of Medicine, Springfield, IL 62794.

• If neurons could be isolated and cultured from adult brain tissue, numerous studies on genetic regulation, development, grafting and aging could be conducted. Cultures can also provide a uniform environment to test for age-related changes or other genetic differences. Here, we describe techniques for the culture of hippocampal neurons from any age rat and apply this capability to determine whether neurons from aged rats are more susceptible to stressors such as glutamate excitotoxicity and lactate acidosis. By dissection of the hippocampus from rats from 0 to 24 months old, we have isolated by digestion with papain and separation on a density gradient an average of 500,000 neuron-like cells per brain (n=17). By linear regression, there was no significant effect of age on the cell yield. By staining with antibodies to neurofilament, greater than 70% of these cells are neuron-like and less than 30% are like glia, staining with GFAP. In three experiments, we compared hippocampal neuron viability from young (3 or 4 months) to old (19 or 24 months) rats after 4 days in culture. In our serum-free B27 in Neurobasal without fibroblast growth factor 2 (FGF), viability was 12% for neurons from young rats and 19% from old rats. The difference is not significant. In the presence of FGF, viability increased to 20 to 60% at 4 days in culture. Good survival continued for at least 3 weeks. Removal of FGF and exposure to lactic acid (1 to 3 mg/ml) for 24 hrs indicated that neurons from a 24 month old rat were significantly more susceptible to acidosis than those from a 3 month old rat. Similar studies with 24 hr treatment with glutamate (50 to 200 µM) resulted in substantial excitotoxicity for neurons from old rats compared to little effect on neurons from young rats in culture. This is the first time aged rat neurons have been cultured for extended periods and shown to exhibit increased sensitivity to stress. These techniques should facilitate studies of the age-dependence of Alzheimer's disease.

673.9

NEURODEGENERATION IN THE SEPTO-HIPPOCAMPAL SYSTEM: EFFECT OF SEPTAL QUINOLINIC ACID INJECTIONS. J.D. Brioni *, A.B. O'Neill and S.J. Morgan. Neuroscience Discovery and Pathology Department, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064-3500.

In many neurological and neurodegenerative disorders, neuronal death can result from the excessive release of endogenous excitatory amino acids like glutamate. As excitotoxicity may constitute a final common pathway for neuronal injury and death, we investigated the dynamics of the toxic effect of another endogenous substance, quinolinic acid, after its injection in the medial septum/diagonal band area (MS/DB) of Long-Evans male rats.

Quinolinic acid (60, 180 and 600 nmoles in 0.5 µl) was injected into the MS/DB area, and histological, biochemical as well as behavioral studies were conducted starting 3 days after the injections. Groups of radiofrequency-lesioned (RF) rats were also included for comparison. Histological evaluation after hematoxylin-eosin staining of paraffin-embedded sections revealed a differential degree of necrosis, glial proliferation and cyst formation in the MS/DB area. The loss of pyramidal neurons in the hippocampus was present only in quinolinic-injected animals and appeared to be time-dependent and dosage dependent. In the behavioral studies, quinolinic acid induced a dose-related ataxia and disinhibition to aversive stimuli in the elevated plus-maze model of anxiety. In the water maze test, an impairment in the processing of spatial information was present without any effect on cue learning. Hippocampal ChAT activity was significantly reduced only in RF-lesioned rats. The neurodegenerative process induced by quinolinic acid injections in the MS/DB area indicate the sensitivity of the septo-hippocampal neurons to this endogenous excitotoxin as demonstrated by histological and behavioral parameters.

673.10

EFFECTS OF A BILATERAL NEUROTOXIC LESION OF THE RHINAL CORTEX ON CEREBRAL GLUCOSE UTILIZATION (CMRglc) IN BABOONS: A PET STUDY. K. Meguro, C. Chavoix, X. Blaizot, C. Le Mestric, F. Hansen, E.T. MacKenzie* and J.C. Baron. INSERM U320, CNRS URA 1829, Centre Cyceron, University of Caen, BP 5229, 14074 Caen, France.

Alterations in the rhinal cortex may play a crucial role in Alzheimer's disease (AD). In AD, the entorhinal cortex (ERh) is, with the hippocampus, the area most affected by neurofibrillary tangles while the cerebral metabolic rate for glucose is most reduced in temporo-parietal associative cortices; these two features are markers for the severity of dementia. Furthermore, combined ablations of ERh and perirhinal cortex (PRh) in macaques leads to a severe declarative memory deficit. To assess the relationships between rhinal alterations and cortical hypometabolism, we studied the effects of stereotaxic ibotenic acid lesions of ERh and PRh on CMRglc in *Papio anubis* baboons. Magnetic resonance imaging (MRI) was used to 1) pre-operatively locate the target sites (n=21-22 per side, 1/2 in each rhinal area), 2) post-operatively confirm the lesion location, 3) determine internal brain landmarks for PET positioning, and 4) identify brain structures for CMRglc measurements. Coronal PET coregistered with MRI was performed under phencyclidine-$N_2$0, once before and 3-4 times after surgery (at days 10-20, 25-32, 39-50, and 74-107). In contrast to sham-operated baboons (n=2), there was a stable and diffuse post-operative decline (10 to 30%) in CMRglc following Rh lesion (n=4). The areas most affected were not only the hippocampus but also the temporal, occipital associative and insular cortices, consistent with the known anatomical connections of the rhinal areas. Declines in CMRglc more or less important than expected (e.g. in basal ganglia and cingulate, respectively) also occured. These preliminary results suggest that severe rhinal neuronal loss (histologically confirmed in two baboons) induces a long-lasting effect on brain metabolism. Relationships between these metabolic effects and memory performance are currently being investigated.

673.11

NON-Aβ COMPONENT OF ALZHEIMER'S DISEASE AMYLOID (NAC) IS AMYLOIDOGENIC. A. Iwai*, M. Yoshimoto, E. Masliah and T. Saitoh. Department of Neurosciences 0624, University of California at San Diego, La Jolla, CA92093-0624

The non-Aβ component of Alzheimer's disease (AD) amyloid (NAC) was identified biochemically as the second major component in the amyloid purified from brain tissue of AD patients. NAC, derived from its 140-amino-acid long precursor, NACP, is at least 35 amino acids long (NAC35) although its amino terminus is not definitely determined. An affinity-purified anti-NAC-X1 antibody against the amino-terminal 9-amino acid sequence of NAC35 immunostained amyloid in AD brain sections and recognized NAC35 but not NACP on Western or dot blot. In aqueous solutions, synthetic NAC35 self-aggregated in a time-, concentration-, and temperature-dependent manner. NAC35 was detected initially as a monomer with a molecular mass of 3500 Da, but became aggregated as a function of time into a higher molecular mass component that could not migrate into the gel. The aggregate of NAC35 showed green-gold birefringence after Congo-red staining when analyzed under polarized light and a fiber-like structure when analyzed ultrastructurally. These results suggest that NAC can form amyloid after it has been cleaved out of its precursor and may be a crucial factor in amyloidosis in the brain. This work has been funded by Alzheimer's Disease Research, a program of the American Health Assistance Foundation, NIH grant AG05131, and was supported by Yamanouchi Pharmaceutical Co., Ltd.

673.12

IDENTIFICATION OF THE DINUCLEOTIDE REPEAT POLYMORPHIC SEQUENCE IN THE NACP GENE. Y. Xia, H.A.R. de Silva, A. Roses, X. Chen, E. Masliah, R. De Teresa, M. Sundsmo, D. Galasko, L. Hansen, R. Katzman, L. Thal, J.M. Roch*, and T. Saitoh Dept. of Neuro-sciences, Sch. of Med., Univ of CA, San Diego, La Jolla CA 92093.

Previous studies have shown the presence of a NAC peptide in amyloid preparations from patients with Alzheimer's disease (AD). We cloned its precursor, NACP, and identified that NACP is the synaptic protein loosely bound to presynaptic vesicles. In the current study, we identified a dinucleotide repeat microsatellite in the NACP gene and its polymorphism. The microsatellite found in a NACP intron contains four clusters of the dinucleotide repeat (CT)10N5(CT)8C(AT)7(AC)12. Amplification of this region of DNA identified five different polymorphisms in Caucasians. Uneven distribution of NACP allele 2 was found among various domestic groups. One-quarter of healthy APOE ε4 carriers have NACP allele 2 whereas only 10% of AD individuals have this allele. We also quantified the NACP protein using Western blots. The result demonstrates that the concentration of NACP is higher by 80% in individuals with NACP allele 2 in AD victims. The higher levels of NACP might indicate that these individuals have higher levels of synaptic reserve, a higher degree of synaptic plasticity, or a higher buffering capacity to sequester toxic Aβ peptide.

673.13

Bax Immunoreactivity In Aged and Alzheimer's Brains. M. Primiano, D. Palm, E. G. Stopa†, R. Corona*#, P. Chan, and C. Johanson. Dept. Clin. Neurosci., Program in Neurosurg. & †Dept. of Pathology, Brown Univ. & RI Hospital, Providence, RI 02903 and #Dept. of Pathology, SUNY Health Sci. Ctr., Syracuse, NY 13210.

Alzheimer's disease (AD) is associated with the accumulation of ß-amyloid protein and the loss of specific neurons. ß-amyloid has been shown to induce neuronal apoptosis *in vitro* and it has been suggested that apoptosis may be involved in Alzheimer's related neurodegeneration. Apoptosis appears to be regulated by the ratio of bcl-2, which promotes cell survival, and bax, which accelerates cell death. Both of these genes are present in neural tissue. We sought to investigate the possibility that changes in the expression of the bax protein in the aging brain may contribute to the progression of Alzheimer's related neurodegeneration. Therefore, we examined the immunohistochemical distribution of bax protein from samples of brain tissue from control and Alzheimer's patients classified with early and severe AD. Sections were blocked with 4% normal goat serum, and incubated overnight at 4°C with a polyclonal rabbit antibody specific for bax (Santa Cruz) or antibody preincubated with the immunizing peptide. Following incubation with biotinylated goat anti-rabbit IgG, sections were incubated with avidin/biotin/peroxidase complex and diaminobenzidine was used as a chromagen. Immunoreactive bax protein was detected within the cytoplasm of a small population of neurons. Additionally bax immunoreactivity was detected within the epithelial cells of the choroid plexus. Glial cells did not appear to be immunoreactive for bax. The number of bax immunoreactive neurons appears to correlate with the severity of AD. These results suggest that the presence of bax protein may be associated with the progression and severity of AD.

Supported by funds from AE10682 and Rhode Island Hospital.

673.14

TRANSPORT OF AMYLOID PRECURSOR PROTEIN (APP) IN HIPPOCAMPAL NEURONS. P.J. Tienari, E. Ikonen, B. de Strooper, M. Simons, A. Weidemann, C. Czech, T. Herdegen*, C. Dotti, and K. Beyreuther. ZMBH and Inst. Physiol., Univ. Heidelberg, Germany and Cell Biology Programme, EMBL, Heidelberg, Germany.

APP is a ubiqutously expressed transmembrane glycoprotein with an as yet unknown function and is the parent molecule of Alzheimer's disease (AD) βA4-amyloid peptide. Mutations in the APP gene have been linked to early-onset AD thus highlighting the importance of brain APP-metabolism in AD. Therefore it is of particular interest to study the transport of this protein in neurons. In order to follow the trafficking of newly synthesised APP in cultured rat hippocampal neurons we used the Semliki Forest Virus expression system and immunofluorescence microscopy. We found that APP is after its initial synthesis first transported into the axon and 30-60 min later is also found in the dendrites. APP-mutations implicated in early-onset AD did not change its initial axonal targeting. However, the axonal transport was greatly reduced when specific portions of the extracellular domain were deleted. These results suggest that the extracellular domain of APP plays an important role in the polarised (axonal vs. dendritic) transport of APP and may provide insights into the pathways and molecular interactions of amyloid formation.

673.15

PURIFICATION AND DETAILED CHARACTERIZATION OF PERLECAN ISOLATED FROM THE ENGELBRETH-HOLM-SWARM (EHS) TUMOR FOR USE IN AN ANIMAL MODEL OF Aß AMYLOID PERSISTENCE IN BRAIN. G.M. Castillo, J.D. Miller, J.A. Cummings, C. Ngo, W. Yang and A.D. Snow*. Dept. of Pathology, Neuropathology Labs, Box 356480, University of Washington, Seattle, WA 98195-6480.

Previously we have demonstrated that coinfusion of perlecan (a specific heparan sulfate proteoglycan) and beta-amyloid protein (Aß) into rat hippocampus leads to a consistent animal model to study the effects of fibrillar Aß amyloid in brain (Neuron 12:219-234, 1994). We have recently observed that the final purity of perlecan may be important to the observed effects in the animal model such that contaminating proteins produced by the EHS tumor may reduce the extent and consistency of congophilic Aß amyloid persistence in brain. In the present study, we describe our method of perlecan isolation, and the stringent testing we employ to ensure the highest possible quality of our perlecan preparations for use in the animal model. Briefly, 50 or 100 grams of EHS tumor tissue is extracted using 4M guanidine-HCl, followed by anion-exchange and gel filtration chromatography. SDS-PAGE followed by staining with silver and Coomassie blue, demonstrate no other contaminating proteins in our perlecan preparations. Western blots using a specific perlecan core protein antibody (HK-102) following heparitinase digestion show a characteristic doublet at 400 and 360 kDa indicative of intact perlecan core protein. Absence of contamination by other basement membrane components produced by the EHS tumor is confirmed by absence of immunoreactive bands on Western blots using antibodies against laminin, fibronectin or collagen type IV. Our procedures for isolation and characterization of perlecan from EHS tumor ensures perlecan of the highest quality, to maximize the potential effects of Aß amyloid deposition/persistence in brain in the animal model.

673.16

DNA FRAGMENTATION IN ALZHEIMER'S DISEASE HIPPOCAMPUS:- CORRELATION WITH TAU AND β-AMYLOID IMMUNOREACTIVITY M. Dragunow*, P. A. Lawlor, R.L.M. Faull and H. Waldvogel. Departments of Pharmacology and Anatomy, School of Medicine, Auckland Univ, Auckland, New Zealand.

A number of recent studies indicate that cells in postmortem brain tissue from Alzheimer's disease cases show in situ evidence of DNA fragmentation, suggesting that these cells are undergoing apoptosis. We have examined hippocampal sections from neurologically-normal and Alzheimer's disease post-mortem cases and compared DNA fragmentation (detected using the TUNEL method) with Tau and β-amyloid immunoreactivity. In addition, we undertook double-label studies to investigate whether TUNEL-positive cells were also Tau, calbindin, calretinin, and/or GFAP positive. Results show that most TUNEL-positive cells in Alzheimer's disease hippocampi are not neurons and there is no strong correlation between the number of TUNEL-positive cells and the number of Tau-positive cells. The implications of these results to mechanisms of cell death in Alzheimer's disease will be discussed. Supported by the NZ Health Research Council.

673.17

EVOLUTION OF β-AMYLOID PLAQUES IN ALZHEIMER'S DISEASE: ROLE OF ASTROCYTIC S100β. R.E. Mrak,* J.G. Sheng and W.S.T. Griffin. Dept. of Veterans' Affairs Med. Ctr., Ark. Children's Hospital Res. Inst., Univ. of Ark. for Med. Sci., Little Rock, AR 72202.

S100β, a neurite extension factor, is overexpressed by activated astrocytes associated with amyloid-containing plaques in Alzheimer's disease and has been implicated in dystrophic neurite formation in these plaques. We sought to correlate S100β-immunoreactive ($S100\beta^+$) astrocytes with the formation of dystrophic neurites and thus with the evolution of diffuse amyloid deposits into neuritic plaques. Dual immunohistochemical labelling was used to determine the number of $S100\beta^+$ astrocytes associated with different plaque types in temporal lobe of 12 Alzheimer patients, age 65 to 88. Amyloid-immunopositive plaques were classified into four types according to the pattern of amyloid distribution (diffuse *vs* dense core) and the presence or absence of β-amyloid precursor protein- (β-APP) immunoreactive dystrophic neurites. Diffuse non-neuritic plaques had small numbers of associated $S100\beta^+$ astrocytes (1.3 ± 0.1 cells/plaque; mean ± SEM; 80% of plaques had associated $S100\beta^+$ astrocytes). In contrast, diffuse neuritic plaques had the most associated $S100\beta^+$ astrocytes (4.2 ± 0.2; 100%), and dense-core neuritic plaques had fewer $S100\beta^+$ astrocytes (1.6 ± 0.2; 90%). Dense core non-neuritic plaque were almost devoid of $S100\beta^+$ astrocytes (0.15 ± 0.05; 12%). Computerized image analysis showed that the number of plaque-associated $S100\beta^+$ astrocytes significantly correlated with the cross-sectional area of plaque β-APP immunoreactivity, an index of the size of the plaques' dystrophic neurite shells ($R = 0.66$, $P < 0.05$). These results are consistent with a role for S100β in the induction and maintenance of dystrophic neurites in plaques in Alzheimer's disease. Supported in part by NIH AG10208, NS27414, and AG12411.

673.18

STRUCTURAL ORGANIZATION OF THE MOUSE APLP2 GENE. K. Paliga[1], H. Mechler[2*], S. Kreger[1], A. Weidemann[1], C.L. Masters[3] and K. Beyreuther[1]. 1) Center for Molecular Biology Heidelberg, INF 282, 69120 Heidelberg; 2) F: Hoffmann-La Roche Ltd, Preclinical CNS Research, Basel, Switzerland; 3) Dept. Pathology, Univ. Melbourne, Parkville, Victoria, 3052 Australia. Recent identification of APLP1 and APLP2, two novel proteins highly homologous to Amyloid Precursor Protein (APP) provides evidence for the existence of a new multigene family. The human APP encoding gene has been localized to chromosome 21 and consists of 18 exons. Tissue specific alternative splicing results in the generation of multiple transcripts differing in the length of the corresponding protein products from 677 to 770 amino acids. As a further step towards understanding the evolutionary relationships between members of the family of APP-like proteins we characterized the exon-intron organization of the mouse APLP2 gene. The 129SVJ mouse genomic library in Lambda FIXII (Stratagene) has been screened using random primed APLP2 cDNA fragments as probes. DNA from plaque purified hybridizing phages has been prepared and analyzed using standard methods. Exon-intron junctions were determined by dideoxy sequencing. As a result of three independent screening rounds we have isolated five lambda phages, which cover almost the entire gene, extending from the 1st intron to the last exon and additional 9 kb of 3'-flanking sequences. In contrary to the human APP gene, which has been estimated to be app. 400 kb long, the mouse APLP2 gene encompasses about 50 kb and consists up to 18 exons. Determination of exon-intron boundaries reveals strong conservation of the overall gene structure between APP and APLP2 underscoring the phylogenetic relatedness of both genes.

ALZHEIMER'S DISEASE: NEURONAL INJURY AND DEATH

674.1

CEREBROVASCULAR INSUFFIENCY: A RISK FACTOR FOR COGNITIVE DECLINE IN AGING AND ALZHEIMER'S DISEASE? P.G.M. Luiten*, G.I. de Jong, C.M. Stienstra, B.T. Stuiver, S. Knollema, J. Korf, K. Majtenyi. Lab. of Animal Physiology, University of Groningen, Haren, The Netherlands.

Although cognitive impairment in aging and Alzheimer's disease (AD) is mainly associated with neuronal alterations, the cerebrovascular system also undergoes prominent changes. With electronmicroscopic (EM) techniques we previously showed progressive decline of the microvascular wall in the cerebral cortex of aged rats. Aged rats show basement membrane thickening, massive perivascular collagen deposits and pericyte degeneration, which suggests a hampered transport fuction of the blood brain barrier.

The importance of the cerebral microvascular condition and blood supply for cognition was studied 1) in AD by EM analysis of the microvascular wall and 2) in adult rats exposed to mild ischemia (unilateral carotic artery occlusion combined with 5 min hypoxia of 10% O_2). Ischemia effects were assessed in spatial learning paradigms like Morris maze and hole board.

In entorhinal and prefrontal cortex in AD and controls a high incidence of microvascular anomalies was observed including basement membrane thickening, collagen deposits and pericyte degeneration. Preliminary data suggest that microvascular pathology is more severe in AD patients.

Neuronal death (after 1-7 days) was almost absent in mild experimental ischemia, but there was a prominent and significant decrease in the learning rate in the initial phase of spatial orientation learning tested 4 weeks after the ischemic episode.

In conclusion, microvascular pathology occurs with high incidence in the aging human brain and appears to be more severe in AD. Short periods of hypoperfusion in rat yield subtle but significant cognitive alterations

674.2

BRAIN LEVELS OF THIAMINE AND ITS METABOLIZING ENZYMES IN ALZHEIMER'S DISEASE. Frank Mastrogiacomo[1], Lucien Bettendorff[2], Thierry Grisar[2], Lori Dixon[1]* and Stephen J. Kish[1]. [1]Human Neurochemical Pathology Laboratory, Clarke Institute of Psychiatry, Toronto, Ontario, Canada, and [2]Laboratory of Neurochemistry, University of Liège, Liège, Belgium

Clinical data suggest that high dose thiamine (Vitamin B1) may have a mild beneficial effect in some patients with Alzheimer's disease (AD). Since the mechanism of this effect could be related to a brain deficiency of thiamine, we measured the levels of free thiamine and its phosphate esters thiamine monophosphate and thiamine diphosphate (TDP), and the activities of three TDP-metabolizing enzymes (thiamine pyrophosphokinase, thiamine diphosphatase, and thiamine triphosphatase) in autopsied cerebral cortex of 18 patients with AD and 20 matched controls. In the AD group mean levels of free thiamine and its monophosphate ester were normal whereas levels of TDP were significantly reduced by 18%-21% in all three cortical brain areas examined. Activities of the TDP-metabolizing enzymes were normal in the AD group, suggesting that decreased TDP is not due to altered levels of these enzymes. The TDP decrease could be explained by reduced levels in AD brain of either ATP, which is needed for TDP synthesis, or energy metabolizing enzymes which utilize TDP as a cofactor and which therefore may act as a "sink" for thiamine storage. Although the magnitude of the TDP reduction is only slight, a chronic subclinical TDP deficiency could contribute to impaired brain function in AD and might provide the basis for the modest improvement by thiamine in cognitive status of some patients with AD. (Supported by US NINDS NS 26034).

674.3

REDUCED PREVALENCE OF DIABETES IN ALZHEIMER'S DISEASE RELATIVE TO OTHER DEMENTIAS. J. H. Nolan, K. A. Nielson, R. A. Mulnard*, C. A. Sandman, and C. W. Cotman. Depts. of Cognitive Science, Psychiatry & Human Behavior, and the Brain Aging and Dementia Institute, University of California, Irvine, CA, 92717.

Diabetes mellitus and Alzheimer's disease (AD) are significant health care problems in the elderly population. The present study examines the coexistence of diabetes and AD in a large, well-characterized patient population, and examines the contribution of vascular disease history to diabetes prevalence among dementia groups.

The assessment records of 275 dementia patients were evaluated by diagnostic group for the existence of diabetes and vascular disease risk factors (hypertension, heart disease, stroke, hyperlipidemia, etc.). Diabetes occurred significantly less often in AD patients than in patients with other dementias. Vascular disease, which is closely linked to diabetes, was more prevalent in AD patients than would be expected given the exceptionally low occurrence of diabetes in this group. Further, there were no differences among the dementia groups in fasting glucose levels.

Therefore, diabetes is very rare in patients with AD. Vascular disease and fasting glucose levels did not explain the reduced prevalence of diabetes in AD. Thus, the disassociation could provide new insight about the pathogenesis of AD. Glucose, insulin sensitivity, and perhaps insulin degrading enzyme may be important factors.

674.4

GENE EXPRESSION OF ND4, A MITOCHONDRIAL DNA-ENCODED SUBUNIT OF NADH DEHYDROGENASE, IS DECREASED IN NEURONS OF ALZHEIMER'S DISEASE BRAIN. R. Fukuyama, J. Fu, K. Hatanpää, C. Jones, D.R. Brady, K. Chandrasekaran* and S.I. Rapoport. Lab. Neurosciences, NIA, NIH, Bethesda, MD 20892

We reported decreased levels of gene expression and enzyme activity of cytochrome oxidase, a mitochondrial enzyme of oxidative phosphorylation, in affected brain areas in Alzheimer's disease (AD). These findings lead us to examine the hypothesis that energy impairment in neurons of AD cause neurodegeneration observed in AD brain. We extended our observations by analyzing the level of gene expression of a subunit of another mitochondrial enzyme, NADH dehydrogenease (complex I of oxidative phosphorylation) in this report. First, we raised monoclonal antibodies (mAbs) against entorhinal cortical grey matter dissected from a normal human brain by applying a modified hybridoma technique (SOFISTIC immunization). One mAb, BG5, immunochemically detected an antigen only in the mitochondrial subcellular fraction separated from other organelles in the rat brain. We further isolated a cDNA clone immunoreactive to mAb BG5 by screening an expression cDNA library and found that the cDNA fragment is a portion of ND4, a subunit of NADH dehydrogenase. We characterized the localization and the level of expression of ND4 in adult brains and cultured cells of rat and human with immunohistochemical and molecular biological procedures. Finally, we compared the level of ND4 gene expression in normal human brains to that in AD brains. We found that ND4 is highly expressed in the cytoplasm of large neurons and that immunohistochemical staining of brain sections showed a significant decrease of ND4 in several brain areas in AD, without a significant decrease in the number of neurons. Northern blot analysis also showed a similar decrease of the expression of ND4 gene in AD. These results indicate that gene expression of mitochondrial DNA-encoded enzymes is generally decreased in AD and suggest that the energy impairment in selective neurons may be involved in neurodegeneration in AD.

674.5

MITOCHONDRIAL MEMBRANE FLUIDITY AND OXIDATIVE DAMAGE TO MITOCHONDRIAL DNA IN AGED AND AD HUMAN BRAIN. P. Mecocci[1]*, M.F. Beal[2], R. Cecchetti[1], M.C. Polidori[1], A. Cherubini[1], F. Chionne[3], L. Avellini[4], G. Romano[1], U. Senin[1]. [1]Gerontology & Geriatrics, Dept. of Clinical Medicine, Pathology and Pharmacology, Perugia University, Perugia (Italy); [2]Neurochemistry Lab, Neurology Service, Massachusetts General Hospital Harvard Medical School, Boston (USA); [3]Department of Medical Physics, Policlinico Monteluce, Perugia (Italy); [4]Institute of Biochemistry and Medical Chemistry, Perugia University, Perugia (Italy).

Oxidative damage on biological molecules has been proposed as a major cause of alterations observed in aging brain as well as in neurodegenerative diseases. In this study we measured membrane fluidity in mitochondria extracted from three cerebral regions and cerebellum of Alzheimer's disease (AD) patients and age-matched controls by means of fluorescence polarization technique. A significant reduction of mitochondrial membrane fluidity was found in AD, except in cerebellum. In controls, a decrease of membrane fluidity was observed along with age and it was also related to the contenent of the oxidized nucleoside 8-hydroxy-2'-deoxyguanosine (OH^8dG) in mitochondrial DNA (mtDNA). Alteration in membrane fluidity seems to be mainly a result of lipid peroxidation since it dramatically decreased when mitochondria were exposed to $FeCl_2$ and H_2O_2. The parallel increase of viscosity in mitochondrial membrane and amount of OH^8dG in mtDNA is suggestive of a relationship between these biological markers of oxidative stress, supporting the hypothesis of a membrane mediated oxidation of DNA. From these results in AD patients biological molecules seem to be particularly sensitive to oxidative stress suggesting that free radicals, together with aging, may play a fundamental role in the pathogenesis of the disease.

674.6

ALZHEIMER'S DISEASE: A HYPOTHESIS ON PATHOGENESIS. D. Harman[1] and J. F. Rodriquez-Sierra[2*]. Dept. of Medicine, Dept. of Cell Biology and Anatomy. College of Medicine., University of Nebraska Medical Center, 600 So. 42nd Street, Omaha, NE 68198-4635, USA.

Senile dementia of the Alzheimer's type (SDAT) is a sporadic systemic disorder whose major manifestations reflect loss of neurons involved in memory. The same pattern of neuronal loss is seen at later ages in normal individuals.

It is hypothesized that SDAT is the result of a mutation in a mtDNA molecule of a non-germ line cell (the progenitor cell) early in development; this increases the rate of cellular aging, by impairing ATP formation and increasing production of superoxide radical and H_2O_2. Clonal expansion of the progenitor cell results in a spectrum of daughter cells; some with many copies of the mutated mtDNA, others may have none. Daughter cells are distributed to the developing organism in such a manner that with advancing age cellular dysfunction and death occurs first in brain areas associated with SDAT.

SDAT incidence may be decreased by maternal antioxidant supplementation and/or increased consumption of fruits and vegetables. SDAT patients may be improved by measures employed for other mitochondrial disorders.

674.7

SUPPRESSIVE EFFECT OF A NON-PEPTIDE PROLYL ENDOPEPTIDASE (PEP) INHIBITOR, Y-29794, ON THE AMYLOIDGENESIS OF SENESCENCE-ACCELERATED MICE (SAM). A. Kato*, A. Fukunari, Y. Sakai, and T. Nakajima. Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd., 871 Fukuoka, Japan.

One of the early symptoms of Alzheimer's disease (AD) is characteristic deposition of ß-amyloid peptide (Aß) among brain regions. Although prevention of this brain amyloidgenesis is thought to be the plausible approach to halt or slow down the progression of AD, the lack of animal models hinders the progress of the research. We, however, previously reported close temporal and spatial relationships between deposited structures of PEP and Aß in SAM hippocampus, suggesting that SAM could be used as an in vivo model for testing therapeutic compounds. The purpose of this study was to estimate whether chronic treatment of SAM with a non-peptide PEP inhibitor, Y-29794, could suppress the progression of amyloidgenesis in the hippocampus.

Male SAMP8/Ta/Sea (senescence-prone) were given oral doses of Y-29794 (1, 10, and 20 mg/kg/day) in drinking water from 2.5 to 8 month-old. Paraffin-embedded coronal sections of the hippocampus were made to stain Aß with polyclonal rabbit anti-Aß(1-16). Aß-like immunoreactivity (Aß-LI) was analyzed by computerized image analyzer and NIH Image.

Deposited structures of Aß-LI which were composed of fine granules were observed in the hippocampus of SAM. After the repeated dosing of Y-29794, the number of granules per deposit, and total number of Aß granules were decreased. Moreover, Y-29794 decreased denser part of characteristic biphasic distribution of the mean density of the granules.

These results indicate that Y-29794 suppresses the amyloidgenesis in the hippocampus of SAM, providing further support of our working hypothesis that PEP is closely involved in the amyloidgenesis, and that Y-29794, an inhibitor of PEP, would be promising as a therapeutic agent for AD.

674.8

CHARACTERIZATION OF A Tc1 INSERTION MUTANT OF *apl-1* IN *CAENORHABDITIS ELEGANS*. J. Daigle* and C. Li. Dept. of Biology, Boston University, Boston, MA 02215.

The amyloid precursor protein (APP) is thought to be involved in Alzheimer's disease, but its specific function is still unknown. We are interested in investigating the function of APP by looking at *APP*-related genes in the nematode *Caenorhabditis elegans*. We have isolated an *APP*-related gene, *apl-1*, from *C. elegans*. The *apl-1* gene is approximately 4.4 kbp, has 12 exons and 11 introns, and has been positioned to the X chromosome of *C. elegans*.

To look at the function of APL-1, a Tc1 transposon insertion mutant in *apl-1* has been isolated by R. Plasterk. The insertion site of Tc1 has been located to the 12th exon, in the region corresponding to the first residue of the transmembrane domain. A strain homozygous for the insertion in *apl-1*, however, showed no phenotype. RT/PCR and Northern blot analysis suggest that the transposon is removed and is not present in the mRNA, as often observed in *C. elegans*. In addition, Western blot analysis showed no size difference between the wild-type protein and the insertion mutant protein, supporting the idea that the transposon does not disrupt gene function in *apl-1*::Tc1 animals. Excision screens are underway to isolate mutants in which Tc1 excision creates a deletion in *apl-1* and, presumably, disrupts its function.

To examine the effects of *apl-1* overexpression, we are producing transgenic animals carrying constructs of the *apl-1* cDNA under the control of a heat shock promotor and a neural-specific promotor.

674.9

BCL-2 PROTEIN IMMUNOREACTIVITY INCREASES IN ALZHEIMER'S DISEASE BRAIN WITH DISEASE SEVERITY. Takao Satou, Brian J. Cummings and Carl W. Cotman*. Institute for Brain Aging and Alzheimer's Disease. University of California, Irvine. Irvine, CA 92717-4550.

Bcl-2 protein has been suggested to be one of the proteins preventing apoptosis in a variety of cell types. Recently, apoptosis has been suggested to have an important role in the pathogenesis of Alzheimer's disease(AD). We have utilized Bcl-2 immunohistochemical methods to examine Bcl-2 in the hippocampus and entorhinal cortex of AD patients ranging in clinical and neuropathological severity from mild to severe and compared these results to those from controls. Immunoreactivity for Bcl-2 was increased relative to controls in most neurons of the entorhinal cortex, subiculum, CA1, CA2, CA3, hilus and dentate gyrus granule cells. Bcl-2 staining increased with increasing disease severity. However, neurons displaying increased immunoreactivity for markers of neurofibrillary tangle formation showed reduced staining for Bcl-2, suggesting that Bcl-2 may be downregulated in these neurons. Immunoreactivity of astrocytes and the vasculature was also increased in AD. These results suggest that Bcl-2 protein may have a role in compensation responses to AD pathology, perhaps affording to the remaining neurons a margin of protection from apoptosis.

674.10

PATTERNS OF NEURONAL DNA FRAGMENTATION DETECTED BY TUNEL IN ALZHEIMER'S DISEASE. R.R. Sukhov*[1,2], J.T. Troncoso[1,2,4], C. Kawas[4], V.E. Koliatsos[1-4]. [1]Neuropathology Laboratory, Dept. of [2]Pathology, [3]Neuroscience, and [4]Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Alzheimer's disease (AD), the most common type of dementia, is characterized by cerebral atrophy, ß-amyloid protein (Aß) deposits/senile plaques, neurofibrillary tangles, and loss of synapses. Although morphometric studies of different areas of the CNS demonstrated loss of pyramidal neurons in neocortical layers III and V in AD, the precise extent and distribution of cortical neuronal loss remains undetermined. In the present study, we used terminal transferase-mediated deoxyuridine triphosphate (d-UTP)-biotin nick end labeling (TUNEL) as a method for detection of fragmented DNA to identify degenerating neurons in the cerebral cortex of individuals with autopsy-confirmed AD and to establish whether similar changes occur in age-matched nondemented individuals. In early stages of AD, we found that TUNEL-labeled neurons are restricted to lamina III of isocortex and entorhinal cortex. In late stages of AD, TUNEL-labeled neurons were found in all layers of the above cortices as well as in the hippocampus. The specificity of TUNEL labeling was supported by the absence of labeling in age-matched and young controls. In conclusion, our findings of the laminar pattern of neuronal degeneration suggest that specific neural systems are involved in the pathogenesis of AD and that the TUNEL method can be used as a method for direct demonstration of neuronal DNA fragmentation in early stages of disease.

674.11

QUANTITATIVE ASSESSEMENT OF APOPTOTIC-LIKE NUCLEI IN HIPPOCAMPAL FORMATION OF ALZHEIMER BRAIN Joseph H. Su*, Aileen J. Anderson and Carl W. Cotman. *Brain Aging and Dementia Institute. University of California, Irvine. Irvine, CA 92717-4550 USA.*

Neuronal death is a prominent feature of Alzheimer's disease (AD) and has been suggested to play a significant role in the expression of dementia in AD and perhaps other age related losses of cognitive function. Recent studies *in vitro* have suggested that apoptosis may have a role in the neuronal cell death associated with AD. We have recently demonstrated the presence and morphological features of apoptotic-like nuclei in AD brain and have shown that such apoptotic nuclei are present in a subset of neurofibrillary tangles in AD brains (NeuroReport 5, 2529-2533, 1994). In this study, we examined the hippocampal formation of AD brains using a TdT-end labeling system (ApopTag, Oncor) on vibratome sections to quantitatively assess apoptotic-like nuclei. The vast majority of apoptotic-like nuclei were located in the grey matter, and were found within neurons. 2/3 of PHF-tau positive neurons were associated with apoptotic-like nuclei. Apoptotic-like nuclei were generally not detected within extracellular neurofibrillary tangles. These results suggest that neuronal cell death may occur without neurofibrillary formation, and neurofibrillary tangle bearing neurons may die by apoptosis.

In conclusion, our data indicate that neuronal DNA damage appears to be more prominent and wide spread in AD than previously realized. This could reflect either a mechanism of cell death, DNA damage/repair mechanisms, or both. In this context, TdT labeling may be an early marker of cellular risk.

674.12

FIBROBLAST GROWTH FACTOR RECEPTORS-LIKE IMMUNOREACTIVITY IN ALZHEIMER'S DISEASE

K. Takami*, A. Matsuo, Y. Aimi, O. Yasuhara[1], I. Tooyama[1] and P.L. McGeer, Kinsmen Lab. of Neurol. Res., Univ. of British Columbia, Vancouver, Canada, V6T 1Z3; [1]Institute of Mol. Neurobiology, Shiga Univ. of Med. Sci., Otsu, Shiga, Japan.

Recent studies have revealed the localization of FGFs in astrocytes, neurons and senile plaques of Alzheimer's disease(AD), indicating their involvement in the pathogenesis of the disease. We report here the immunohistochemical localization of FGF receptors (FGFR2, 3 and 4) in AD and control brain tissue, using rabbit polyclonal antibodies (Santa Cruz Biotechnology Inc.). The epitopes recognized by these antibodies in each case mapped to the carboxy terminal region of the human FGFRs. In cerebral cortices of both AD and controls, astrocytes, especially in white matter, showed FGFR2 and FGFR4-like immunoreactivity. Some neuronal fibers also showed FGFR2 or FGFR4 immunoreactivity. Reactive astrocytes having FGFR4-like immunoreactivity were observed around senile plaques. FGFR3-like immunoreactivity was localized to tangles and dystrophic neurites in or near senile plaques.

These findings suggest that FGFs may participate in many functions associated with the pathology of AD through their various receptors, which are distinctly localized.

674.13

TRANSPLANTED SYNGENEIC RAT FIBROBLASTS GENETICALLY MODIFIED TO EXPRESS HUMAN NERVE GROWTH FACTOR ARE BIOACTIVE FOR AT LEAST 6 WEEKS AS DEMONSTRATED BY ENHANCED SURVIVAL OF MEDIAL SEPTAL CHOLINERGIC NEURONS AFTER FIMBRIA-FORNIX TRANSECTION R. J. Mandel*, D. G. Clevenger, D. Nagy, T. M. Jaret, M. Morten, and S. E. Leff. Somatix Therapy Corp. 850 Marina Village Pkwy., Alameda, CA 94501.

This experiment was designed to investigate the duration of bioactivity of intracerebrally grafted transgenic hNGF producing fibroblasts by transplanting the fibroblasts at various time-points **before** fimbria-fornix (FF) lesioning. Syngeneic Fischer dermal fibroblasts were grown in culture (≈ 8 passages) and transduced with a retroviral vector encoding the hNGF cDNA or *E. Coli lac-Z* both driven by the internal viral LTR promoter. *In vitro* hNGF production determined by an ELISA assay (257 ng/10^6 cells/24 hr) was comparable to that reported previously (Rosenberg *et al., Science* 242:1575-1578, 1988). Each rat received 2 grafts of 1 µl of 7.5 X 10^4 of hNGF (n=5-7) or lac-z producing cells (n =5-7) in the medial septum (MS). Immediately following the implantation surgery (day Ø) or following variable delay intervals (4,7, 14, 28 & 42 days), an unilateral FF lesion was performed ipsilateral to the cell implantation. The rats were sacrificed 21 days after the FF lesion. Fixed tissue was sliced at 40 µm and prepared for choline acetyltransferase (ChAT in MS) immunohistochemistry , acetylcholinesterase (AChE) histochemistry (hippocampus), and hemotoxylin and eosin staining. Some animals were removed from the study prior to ChAT+ cell counting by evaluating their FF lesion via examination of the AChE stained sections of the hippocampus. Each MS section was evaluated "blind" to treatment by counting ChAT+ cells and by noting whether the graft was innervated by ChAT+ fibers. There was no effect of surgery delay on ChAT+ cell survival in the lac-z group, therefore the lac-z data were combined into one control group for further analysis. hNGF-producing fibroblasts significantly enhanced survival of ChAT+ neurons at all FF lesion delays ($p < 0.05$) but the magnitude of the survival effect appeared to wane over time (42 day percent survival < 14 day survival, $p < 0.05$).

ISCHEMIA: ISCHEMIC TOLERANCE AND STRESS PROTEINS

675.1

BCL-2 EXPRESSION AND TOLERANCE FOR POSTISCHEMIC NEURONAL DEATH IN THE GERBIL HIPPOCAMPUS.

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Pretreatment with brief (2 min) ischemia induced tolerance for subsequent ischemia (5 min) and prevented delayed neuronal death (DND). Using monoclonal antibody against bcl-2 oncoprotein we previously reported that immunoreactivity of bcl-2 greatly increased in the gerbil hippocampal CA1 sector in parallel with acquisition of tolerance for DND (Shimazaki et al. Neurosci. Res. 20:95,1994). To determine time course and locus of bcl-2 production after sublethal brief ischemia, we have studied mRNA expression in the gerbil hippocampus by *in situ* hybridization. The level of bcl-2 mRNA was markedly increased at 2 days following 2 min ischemia. Northern blot analysis supported the result showing 2-fold increase in bcl-2 mRNA after 2 min ischemia. In the gerbil treated with 5 min ischemia, no increase in the level of bcl-2 mRNA was found. The results suggest close correlation between acquisition of tolerance for DND and the expression of bcl-2, which is known to support survival of the neuronal death.

675.2

TRANSIENT FOCAL ISCHEMIA ELEVATES *bcl-2* EXPRESSION IN THE NEOCORTEX. Z.G. Huang*, A.M. Buchan and G.S Robertson[1]. National Research Council of Canada, and [1]Department of Pharmacology, Faculty of Medicine, University of Ottawa, Ottawa, Canada

Several lines of evidences suggest that apoptotic mechanisms may contribute to neuronal death following transient cerebral ischemia. Over-expression of the proto-oncogene *bcl-2* in several cell lines has recently been shown to reduce apoptotic death produced by a variety of noxious treatments. This finding suggests that there may be a positive relationship between *bcl-2* expression and resistance to ischemic cell death *in vivo*. The present study addressed this hypothesis by examining the effects of transient forebrain ischemia on *bcl-2* expression in the neocortex. Reversible focal cerebral ischemia was produced in male spontaneously hypertensive rats by 90 min of transient right cerebral artery occlusion (MCA-o) combined with permanent right common carotid artery occlusion. Six groups, composed of 4 animals each, were subjected to 90 min of MCA-o and sacrificed after 1, 4, 8, 12, 24 or 48 hr of reperfusion (RP). A seventh group served as sham-operated controls and underwent the same operative procedures except that the MCA was not occluded. Brains were rapidly removed, quickly frozen and sections 12 µm thick cut through the cortex. *bcl-2* mRNA was detected by *in situ* hybridization histochemistry and quantified by computer assisted image analysis. *bcl-2* mRNA levels were low in the neocortex of sham animals. Transient MCA-o elevated *bcl-2* mRNA levels selectively in the ischemic penumbral region of the neocortex within 8-12 hr with peak increases occurring between 12-24 hr of RP. *bcl-2* mRNA levels were still elevated in the penumbra 48 hr after RP. These results suggest that elevated *bcl-2* expression may contribute to the survival of neurons located in the penumbral region following cerebral ischemia.

675.3

The Distribution of bcl-2 Protein In The Rat Hippocampus Following Transient Forebrain Ischemia. N. Knuckey*, M. Primiano, D. Palm, M. Guglielmo, M. Epstein, and C. Johanson. Dept. Clin. Neurosci., Program in Neurosurg., Brown Univ. & RI Hospital, Providence, RI 02903

There is increasing evidence for the involvement of apoptosis in the selective, delayed neuronal death resulting from cerebral ischemia. Bcl-2 is present in the developing and adult nervous system and has been shown to prevent apoptosis and neuronal cell death under many neurotoxic conditions implicated in ischemia. To investigate the possible relationship between bcl-2 and selective ischemic neurodegeneration, we examined the distribution of bcl-2 in the rat hippocampus following transient forebrain ischemia (TFI).

TFI was induced in normothemic adult rats by bilateral carotid artery occlusion with hypotension. Animals were perfused with 4% paraformaldehyde 6, 12, 18, 24, 48, 72 hours following induction of TFI. Frozen 10 µm coronal sections were analyzed for the distribution of bcl-2 protein by immunohistochemistry. Sections were immunostained using a polyclonal rabbit antibody specific for bcl-2 (Santa Cruz) or antibody preincubated with the immunizing peptide. Following incubation with biotinylated anti-rabbit IgG, sections were incubated with avidin/biotin/peroxidase complex and DAB was used as a chromagen.

Immunoreactive bcl-2 was detected within the cytoplasm of most hippocampal neurons including the vulnerable CA1 pyramidal cells of non-ischemic animals and during the first 18 hours following TFI. At approximately 24 hours of recovery, a depletion of immunoreactive bcl-2 was observed in most vulnerable CA1 pyramidal neurons while immunoreactive bcl-2 continued to be present at all recovery times in resistant neurons of the other CA regions and dentate gyrus. These results suggest that the presence of bcl-2 protein may influence neuronal survival following TFI.

Supported by funds from Rhode Island Hospital.

675.4

BCL-2 PROTECTS NEURAL CELL MITOCHONDRIA FROM Ca^{2+} OVERLOAD AND Ca^{2+}-INDUCED RESPIRATORY INHIBITION. A.N. Murphy*, D.E. Bredesen[1], and G. Fiskum. Dept. of Biochemistry and Molec. Bio., George Washington Univ. Medical Center., Washington, D.C. 20037, and [1]La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Recent data suggest that Bcl-2 expression provides neuroprotection in models of cerebral ischemia. Although evidence suggests that Bcl-2 can act as an antioxidant and may affect patterns of Ca^{2+} metabolism, the exact mechanism is unknown. The effect of Bcl-2 expression in GT1-7 neural cells on mitochondrial resistance to damage induced by high concentrations of extramitochondrial Ca^{2+} has been examined. Bcl-2 appears to have little effect on state 3, state 4, or uncoupler-stimulated rates of respiration of digitonin-permeabilized cells under control conditions. However, the addition of 7.7 nmoles of $CaCl_2/10^6$ cells inhibited uncoupler-stimulated respiratory rates of permeabilized control cells by approx. 83%, whereas this rate in Bcl-2 expressing cells was inhibited by only 30%. Measurements of maximal Ca^{2+} uptake capacity prior to Ca^{2+} release revealed that control mitochondria could sequester 600 and 500 nmoles $Ca^{2+}/4.3x10^7$ permeabilized cells respiring on glutamate/malate or succinate, respectively; whereas Bcl-2 expressors could accumulate 1013 and 800 nmoles under the same conditions. In addition, mitochondria isolated from Bcl-2 expressors were significantly less sensitive to respiratory inhibition in response to 42-167 nmoles Ca^{2+}/mg mitochondrial protein. The ability of mitochondria of Bcl-2 expressing cells to maintain function under conditions of cytotoxic levels of extramitochondrial Ca^{2+} may provide a mechanism by which Bcl-2 inhibits delayed neuronal death following cerebral ischemia. (Supported by NIH NS34152)

675.5

REGULATION OF GENE EXPRESSION BY O_2 DEPRIVATION IN *DROSOPHILA MELANOGASTER*. Enbo Ma and G. G. Haddad*. Departments of Pediatrics and Cellular & Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520

We have recently shown that the adult fruit fly *Drosophila melanogaster* (DM) is very tolerant to complete lack of O_2 and can totally recover from 4 hrs of anoxia. To study the mechanisms that are activated during O_2 deprivation and which are potentially protective, we used two groups of DM: one was kept in 0% O_2 for 4 hrs and another was used as a control. Flies from both groups were immediately frozen in liquid N_2, shaken vigorously, and passed through a number of sieves of successively smaller pore sizes in order to collect heads. Poly-A RNA was isolated from both groups of DM heads and the transcripts for heat shock proteins (HSP70 and HSP26), ubiquitins (UB3 and UB4), cytochrome oxidase (COX) and superoxide dismutase (SOD) were quantitatively analyzed by Northern and slot blot analysis. Gene expression for HSP70 was up-regulated by ~3.5 times while UB4 and COX were down-regulated to about half of control during anoxia. HSP26, UB3 and SOD did not change significantly. From these results we conclude that during O_2 deprivation 1) there is differential regulation of gene expression and 2) the up-regulation of HSP70 and down-regulation of UB4 are consistent with the idea that such changes potentially protect against protein degradation. (Supported by NIH T32HL077778, PO1 HD32573, HL28940 and NS32578).

675.6

AN OPTIMIZED MODEL OF INDUCED ISCHEMIC TOLERANCE IN THE GERBIL. H. Abe and T.S. Nowak, Jr.* Dept. of Neurology, University of Tennessee, Memphis, TN 38163.

Brief insults moderate the damage seen following subsequent more severe ischemic challenges, but reported effects are highly variable. In the present study we monitored hippocampal DC potentials to determine effective durations of priming insults and test challenges, identifying threshold depolarization intervals for tolerance induction and changes in gene expression. Ischemia of varied duration was produced by bilateral carotid artery occlusion in halothane-anesthetized gerbils (n=125) fixed in a stereotaxic frame. Hippocampal DC potentials were recorded via glass microelectrodes. Induced mRNAs were evaluated by in situ hybridization at 1 h or 6 h recirculation. In other gerbils a test insult was administered 2 d after the first and consisted of depolarization for ≥385 sec, which produced maximal neuron loss in naive animals. Histological evaluation of CA1 was done 7 d after the test insult. Tolerance was first evident after ≥90 sec depolarization, with complete protection after ≥150 sec. The mRNA encoding the stress protein, hsp72, was not increased after depolarizations ≤120 sec, with consistent induction only after ≥200 sec, and was strongly expressed only after ≥240 sec, which was the threshold for histological damage. In contrast, slight increases in c-fos, junB and junD were detected after the shortest depolarizations (60 sec) and these mRNAs were reliably induced in all hippocampi after ≥120 sec. These results: 1) indicate that the stress response is not required for induced ischemic tolerance, 2) demonstrate that diverse cascades of altered gene expression must occur after insults that induce tolerance, and 3) establish a highly reproducible model in which to study neuroprotective mechanisms after repeated insults.

675.7

HSP70 AND C-FOS mRNA INDUCTION IN THE HIPPOCAMPUS FOLLOWING THE FOCAL ISCHEMIA IN GERBILS: THE RELATIONSHIP TO ISCHEMIC TOLERANCE. K. Miyashita*, T. Nakajima, H. Abe, M. Nishiura, T. Sawada and H. Naritomi. Cerebrovac. Division, Dept. Med. **National Cardiovascular Center**, Suita, Osaka 565, JAPAN.

In gerbil model of unilateral middle cerebral artery occlusion (MCAO), in which both sides of hippocampus are spared from direct ischemic insult, hsp70 and c-fos mRNA induction in the hippocampus was studied using in situ hybridization method. Ischemic tolerance of the hippocampus was also investigated inducing transient forebrain ischemia at 3-7 days after MCAO. Induction of hsp70 mRNA was faint in both hippocampal regions at 12 to 24 h after left MCAO, while c-fos message was clearly induced in the left hippocampus at 0.5 to 6 h following ischemia. Animals undergoing left MCAO displayed ischemic tolerance at 5 min bilateral carotid artery occlusion (5BCO) done at 3 days after MCAO. More than 40% of CA1 neurons survived in the left hippocampus. In contrast, less than 10% of those survived in the right. No such tolerance was observed at 5BCO done at 7 days after MCAO. The dissociation of hsp70 and c-fos mRNA induction after MCAO may be derived from the difference of threshold between these two genes. Hsp70 appears to play little role in the induction of focal ischemic tolerance in the gerbil MCAO model.

675.8

SPREADING DEPRESSION INDUCES TOLERANCE TO ISCHEMIA IN THE CEREBRAL CORTEX OF RATS. S. Kobayashi, V. A. Harris, F. A. Welsh* Div. Neurosurg., Univ. Penna. Sch. Med., Philadelphia, PA 19104.

The objective of the present study was to determine whether cortical spreading depression (CSD) induces tolerance to ischemia in the cerebral cortex of rats. CSD was evoked by applying 2 M KCl to the intact dura over the frontal cortex of one hemipshere for 2 hr. After recovery for 24 hr, bilateral forebrain ischemia was produced using carotid artery occlusion and arterial hypotension. Following ischemia for 6 min (n=7) or 10 min (n=6), animals were permitted to survive for 4-6 days prior to assessment of histopathology. In separate animals (n=5), KCl evoked 11 ± 3 DC shifts and triggered the expression of c-*fos* mRNA, but not hsp72 mRNA, in the ipsilateral cortex. Forebrain ischemia of 6-min duration caused selective neuronal necrosis in many brain areas, but the number of necrotic neurons in the cerebral cortex preconditioned with CSD was significantly lower than that in the contralateral cortex. The extent of neuronal necrosis in striatum and hippocampus was not significantly different between hemispheres. Forebrain ischemia of 10-min duration caused a severe degree of neuronal necrosis and scattered microinfarction. The extent of infarction in the cerebral cortex preconditioned with CSD was markedly diminished relative to that in the contralateral cortex. These results demonstrate that CSD induces tolerance to both mild and severe ischemia in cerebral cortex by mechanisms unrelated to hsp72.

675.9

THE EXPRESSION OF BFGF INCREASED AT TRANSCRIPTION LEVEL BUT NOT AT TRANSLATION LEVEL IN THE CA1 PYRAMIDAL CELL LAYER IN ISCHEMIA-TOLERANCE INDUCED RAT MODEL.

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We previously reported that 10 minutes of transient forebrain ischemia induces bFGF mRNA but not bFGF protein in the CA1 pyramidal cells. In this study, we investigated the changes of bFGF following 2 minutes of transient forebrain ischemia, that is so called ischemia-tolerance induced rat. Transient forebrain ischemia was produced with 4 vessel-occlusion method. *In situ* hybridization demonstrated that 2 minutes of transient forebrain ischemia induced bFGF mRNA in the CA1 pyramidal cell layer by 24 hours after ischemia. But immunohistochemistry failed to show the expression of bFGF in the CA1 pyramidal cell layer. Other investigators have recently reported that ischemia induced bFGF protein in neurons shortly after ischemia *in vitro*. However, our results do not suggest that bFGF plays a key role in the protection for neurons in ischemia-tolerance induced rat.

675.10

ISCHEMIC PRECONDITIONING CONVEYS HISTOLOGICAL BUT NOT FUNCTIONAL PROTECTION.

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Previous studies have shown that prior exposure to brief periods (e.g. 1.5-2 min) of ischemia protects CA1 neurons from a subsequent, lethal ischemic insult several days later. It is not known if this ischemic "tolerance" translates into functional preservation since a number of treatments maintain neurons in a viable, but abnormal state.

Gerbils were exposed to 2 x 1.5 min episodes of global ischemia (24 hr apart) followed 3 days later by a 5 min occlusion that typically destroys >95% of CA1 neurons. In one experiment, animals were tested in an open field test of habituation 3, 7 and 10 days after the last ischemia. In a second experiment CA1 field potentials (fEPSP's)were recorded from hippocampal slices taken 4 days after the last ischemia.

CA1 cell counts showed that preconditioning resulted in about 70% preservation assessed 10 days after ischemia. In spite of the substantial degree of histological protection, open field activity levels in preconditioned gerbils were no different than ischemic gerbils, indicating a marked habituation deficit. Similarly, CA1 fEPSP's were less than 50% of those recorded in sham animals. These data suggest that while ischemic preconditioning protects CA1 neurons histologically, many of these neurons are functionally abnormal.

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675.11

CHRONIC HYPOXIA INCREASES THE NEURONAL RESPONSE TO ACUTE HYPOXIC STRESS.

J.P. O'Reilly* and G.G. Haddad. Depts of Biology, Pediatrics, and Cellular and Molecular Physiology, Yale University and School of Medicine. New Haven, CT. 06520

Acute oxygen deprivation can have severe consequences in the central nervous system (CNS). However, much less is known about the effects of long-term (chronic) hypoxic exposure on the CNS. We hypothesized that exposure to chronic hypoxia would be detrimental to the CNS. We exposed rats (P0-P2) to FiO_2 of 9.5±1% until P21-P35 (*exposed*). Membrane potential (Vm) and input resistance (Rm) were measured during *in vitro* acute hypoxia at two levels of tissue PO_2 in neocortical neurons (NCX) and hypoglossal neurons (XII) using the brain slice technique and intracellular recordings. Age-matched normoxic animals were used as controls (*naive*). In response to acute hypoxia (15 Torr), exposed NCX (n=13) depolarized to a much greater extent (53.2±7.0 mV; ±SE) and showed a larger decrease in Rm (-76.7±8.2%) than naive NCX (n=8; ΔVm=10.6±2.1 mV; $p<0.001$; ΔRm= -17.0±4.6%, $p<0.001$). In response to anoxia (0 Torr), exposed NCX (n=18) showed anoxic depolarization (>20mV/min) much sooner (4.8±0.4 min) than naive NCX (8.8±1.0 min, n=19; $p<0.001$). Exposed (n=25) and naive (n=33) XII showed similar responses to both hypoxia and anoxia. Re-oxygenation produced a rapid recovery of both Vm and Rm in naive NCX and XII, and also in exposed XII. However, exposed NCX remained depolarized during the recovery period (10min) following both hypoxia and anoxia, and Rm showed a delayed recovery pattern. In addition, 28% of the exposed NCX failed to recover from anoxia. We conclude that chronic hypoxic exposure renders neurons in the NCX more sensitive to the effects of acute hypoxia, and possibly more vulnerable to stress-induced injury. (Supported by HD 32573, HL 28940, NS 32578)

675.12

ALTERED NEURONAL PROPERTIES IN NEOCORTEX FOLLOWING LONG-TERM EXPOSURE TO HYPOXIA.

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Neurons in the central nervous system (CNS) are extremely sensitive to oxygen availability in the environment, and oxygen deprivation can have severe physiologic and pathophysiologic consequences. Since little is known about the effects of long-term O_2 deprivation on the developing CNS, we studied neurons from animals chronically exposed to a hypoxic environment. We hypothesized that chronic hypoxic stress would be detrimental to the development of the CNS. Neonatal rats (P0-P2) were raised in a plexiglass chamber with FiO_2 of 9.5 ± 1% (*exposed*). At P21-P35, the animals were removed from the chamber, and the neocortex (NCX) was prepared for morphologic and electrophysiologic study. Golgi stained sections (100 μm) were used to count spine density on apical dendrites of pyramidal neurons in NCX. Data from 6 exposed and 6 age-matched normoxic controls (*naive*) revealed a significant decrease ($p<0.01$) in the mean # of dendritic spines in the NCX from exposed (40.7 ± 1.7; ±SEM) vs naive animals (49.4 ± 1.3). Intracellular recordings from brain slice were used to measure membrane potential (Vm) and input resistance (Rm). Baseline Vm was not different between exposed and naive NCX, while Rm was greater ($p<0.01$) in exposed (53.5 ± 3.4 MΩ, n=41) compared to naive (42.8 ± 2.3 MΩ, n=52). We conclude that 1) long-term exposure to a reduced oxygen environment alters neuronal cytoarchitecture and membrane properties in NCX, and 2) these changes may play a role in the increased response to acute hypoxia in NCX neurons from exposed animals. (Supported by HD 32573, HL 28940, NS 32578)

675.13

"ISCHEMIC TOLERANCE" IN CORTICAL CELL CULTURE.

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Several studies in recent years have established the phenomenon of "ischemic tolerance" in animal models of brain ischemia, where mild ischemic insults render the brain resistant to injury induced by a subsequent, more severe insult (Kitagawa, *Br. Res.* 528:21, 1990; Kirino, *J. Cereb. Blood Flow Metab.*11:299, 1991). This phenomenon is thought to reflect protective cellular alterations induced by the first, preconditioning ischemic insult. The present study was performed to see if the phenomenon could be demonstrated in cell cultures.

Murine cortical cell cultures containing both neurons and glia (DIV 13) were exposed to sham wash, or periods of oxygen-glucose deprivation (5-20 min) too brief to induce detectable cell death. 24 hrs later, these cultures were exposed to a 50-75 min period of oxygen-glucose deprivation, sufficient to induce intermediate levels of neuronal death without glial death in control cultures (as assessed 24 hrs later by morphological examination and LDH efflux to the bathing medium). Cultures preconditioned by exposure to a 20 min period of oxygen-glucose deprivation exhibited 30-70% less neuronal death than those preconditioned by sham wash alone. These experiments support the idea that sublethal ischemic insults can render cortical cells resistant to subsequent ischemic insults, and provide encouragement for further examination of this phenomenon in the culture system.

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675.14

HYPOXIA TRIGGERS NEUROPROTECTIVE ALTERATIONS IN HIPPOCAMPAL GENE EXPRESSION VIA A HEME-CONTAINING SENSOR.

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Pre-exposure of rat hippocampus to short periods of hypoxia increases the resistance of CA1 pyramidal neurons to longer, normally fatal hypoxic insults. We have previously shown that the induction of this neuroprotection requires ongoing RNA and protein synthesis. We hypothesized that, similar to peripheral erythropoeisis, a low [O_2] environment may convert a heme-containing sensor to its deoxygenated conformation, triggering the expression of neuroprotective gene products. Carbon monoxide (CO) can replace O_2 bound to heme iron and effectively lock the porphyrin ring in its oxygenated state. Therefore, we applied 10% CO to hippocampal slices during severe hypoxia, to determine if it could prevent the induction of neuroprotection by hypoxia.. We used a bipolar electrode to stimulate Schaffer collateral axons in area CA1 every 60 s, and recorded evoked dendritic population epsps in stratum radiatum. Naïve slices subjected to a 15 min period of severe hypoxia (95% N_2/5% CO_2) show little or no recovery of synaptic transmission. In contrast, 5 min preconditioning hypoxia markedly improved resistance to subsequent, longer hypoxic episodes. In contrast, when a 5 min preconditioning hypoxia was given in the presence of CO (85% N_2/5% CO_2/10% CO), the induction of neuroprotection was completely blocked. As a control, another group of slices were subjected to 5 min preconditioning hypoxia, followed 1 hour later by a second hypoxia in the presence of CO.. This group of slices exhibited normal resistance to hypoxia-induced loss of synaptic transmssion, indicating that the blockade of neuroprotection by CO could not be attributed to nonspecific toxicity, but are probably due to inactivation of a heme-containing O_2 sensor.

675.15

EFFECTS OF SEVERE ISCHEMIA-HYPOXIA ON GENE EXPRESSION IN YOUNG RATS. K.L. Gilby*, K.M Murphy, J.N. Armstrong, R.W. Currie and H.A. Robertson. Laboratory of Molecular Neurobiology, Depts. of Pharmacology and Anatomy & Neurobiology, Dalhousie Univ., Halifax, NS, Canada, B3H 4H7.

Ischemic brain injury leads to a destructive cascade of events that culminates in the death of certain neurons in hippocampus, striatum and cortex. The mechanism by which this occurs is poorly understood, but one of the early events following injury is a rapid increase in the expression of immediate-early genes and stress genes such as the inducible *hsp-70*. In order to understand the progression of changes initiated by ischemic insult that eventually lead to cell death, we used young rats in the Rice-Vannucci model of ischemia-hypoxia and have studied the effects of severe (60 min hypoxia) injury on the induction of *c-fos*, *c-jun* and *hsp-70* in the hippocampus. Rats (100) were divided into 4 groups (naive control, hypoxic control, ligated control and ligated hypoxic). Two animals that had seizures were excluded from the study. No cell death or increased protein expression was seen in any of the control groups. However, in the ligated hypoxic group, severe damage was observed. Such severe injury was associated with increased expression of the c-Fos and c-Jun proteins on the side ipsilateral to the injury at 12 hrs. However, at 24 hrs the expression of both c-Fos and c-Jun proteins on the ipsilateral side had returned to basal levels while their expression was increased on the contralateral side. HSP-70 levels increased on the ipsilateral side in CA1 at 24 hrs, but not at 12 hrs. At 48 hrs, the levels of c-Fos and c-Jun are at basal levels, but HSP-70 remains elevated on the ipsilateral side. At no time was HSP-70 protein expressed on the side contralateral to the insult. These findings suggest that even in this severe type of ischemic insult, expression of *c-fos*, *c-jun* and *hsp-70* may be involved in the events leading to cell death. Altering expression of these genes may therefore be expected to change the degree of injury. (supported by the MRC and SmithKline Beecham Pharma Inc.)

675.16

NEURONAL AND GLIAL EXPRESSION OF INDUCIBLE HSP70 IN FOREBRAIN ISCHEMIA. D. Xu[1], J.-C.L. Plumier[1], H.A. Robertson[2] and R.W. Currie[1]* Laboratory of Mol. Neurobiology, Depts. of Anatomy & Neurobiology[1] and Pharmacology[2], Dalhousie Univ., Halifax, N.S., Canada, B3H 4H7.

Although induction of HSP70 in neurons in the vulnerable regions in the brain after cerebral ischemia has been well documented, the relationship of the expression with neuronal death and survival remains unclear. In the present study, we examined neurons as well as glial cells for cell death using cresyl violet staining, and for expression of HSP70 by immunohistochemistry at 1.5, 3, 6, 12, 24, 48, 72, and 120 hours after 30 minutes of global ischemia. Immunoreactivity against HSP70 was first observed at 12 hours in neurons in layer V of the cortex, and at 24 hours in layers II and III. Layer VI of the cortex rarely revealed expression of HSP70. Most of the pyramidal cells in the hippocampus expressed HSP70 at 24 hours and the expression remained at high levels until 72 hours. Elevated expression of HSP70 remained until 120 hours in the CA3 region. This pattern of HSP70 expression was seen when there was only moderate cell loss in CA1 at 72 and 120 hours as revealed by cresyl violet staining. However, in some cases, cresyl violet staining revealed abrupt and massive neuronal loss due to cell death in CA1 at 24 hours and in CA3 and dentate gyrus at 72 hours. In these brains, at 24 hours, HSP70 was not observed in CA1 but CA3 had a pattern similar to brains with only moderate cell loss in CA1. Only in cases with severe cell loss, was HSP70 expression seen in layer VI of the cortex. HSP70 expression was observed in glia-like cells, often in clusters, in areas in cortex and hippocampus where neuronal HSP70 immunoreactivity was no longer detected and cresyl violet staining revealed loss of neurons. These glial-like cells were immunoreactive for OX-42. These findings suggest a relationship between neuronal expression of HSP70 and neuronal survival. Glial expression of HSP70 may be associated with activation of the glial cells in response to neuronal necrosis.
(Supported by the MRC of Canada and SmithKline Beecham Pharma Inc.)

675.17

EFFECTS OF CEREBRAL INJURY ON TRANSGENIC MICE EXPRESSING THE HUMAN HSP70. J.-C.L. Plumier[1]*, J.N. Armstrong[1], H.A. Robertson[2], R.W. Currie[1], C.E. Angelidis[4], H. Kazlaris[3], G. Kollias[3], and G.N. Pagoulatos[4], Laboratory of Molecular Neurobiology, Departments of Anatomy & Neurobiology[1] and Pharmacology[2], Dalhousie University, Halifax, N.S., Canada B3H 4H7; Department of Molecular Genetics[3], Hellenic Pasteur Institute, Athens, Greece 11521; Laboratory of General Biology[4], University of Ioannina Medical School, Ioannina, Greece 45332.

In the brain, expression of the inducible HSP70 is well described following many types of injury, including ischemia and kainic acid treatment. This induced expression of HSP70 has been suggested to be associated with neuronal protection to ischemic injury. Recently, we reported that constitutive overexpression of the human inducible 70-kDa heat shock protein in mice increased the contractile recovery of isolated hearts following global ischemia. The protective effect of overexpression of the human HSP70 transgene in mouse brain has not yet been determined. Transgenic mice overexpressing the human HSP70 under the control of the human β-actin promoter were examined for neuronal protection to cellular injury. Presence of the human transgene in brain was determined by S1 nuclease mapping using a fragment derived from the PAT-HS70 plasmid as probe. Western analysis demonstrated constitutive expression of the human HSP70 in mouse brains. In preliminary experiments, kainic acid was injected intraperitoneally into control and transgenic mice. Kainic acid administration (35 mg/kg) lead to 50% death of control mice (n=6) and 89% death of transgenic mice (n=9) during epileptic seizure. This result suggests that overexpression of the human HSP70 transgene in mouse brain does not protect and may even sensitize the hHSP70 transgenic animals to kainic acid induced seizures. We are currently examining these transgenic mice for resistance to ischemic injury.
(Supported by Heart & Stroke Foundation of New Brunswick, MRC of Canada and SmithKline Beecham Pharma Inc.)

675.18

EXPRESSION OF hsp70 mRNA FOLLOWING TRANSIENT BILATERAL COMMON CAROTID ARTERY OCCLUSION IN TRANSGENIC MICE OVEREXPRESSING CuZn-SUPEROXIDE DISMUTASE. Murakami K. [1] Kondo T. [1] Honkaniemi J. [3] Mikawa S. [1] Chan T. [1] Chen S *. [1] Sharp F R. [3] Epstein C J. [2] and Chan P H. [1] (Department of Neurology, Neurosurgery[1] and Pediatrics[2], University of California, San Francisco, CA94143 and Department of Neurology[3] VA Medical Center, San Francisco, CA94121.)

Various studies have demonstrated an increase in heat shock protein 70 (HSP70) synthesis in the brain following transiently induced ischemia, suggesting a protective role for HSP70 against ischemic insult. Recently, it has been reported that HSP70 mRNA (hsp70) expression is affected by oxidative stress. However, the underlying mechanism is unclear. We used transgenic (Tg) mice which overexpress CuZn-superoxide dismutase (CuZn-SOD), to examine the amount and distribution of hsp70 expression following transient bilateral common carotid artery (CCA) occlusion. Male heterozygous Tg mice (Tg HS/SF-218-3) and nontransgenic (nTg) normal littermates (35±4 g) were anesthetized with methoxyflurane. Bilateral CCA were occluded for 3 min using small surgical clips. The mice were decapitated, and brains were frozen and sectioned 20 μm thick. The sections at 4, 24, 72, and 168 h after the surgery were processed for in situ hybridization with [^{35}S]-labeled oligonucleotide probe of hsp70 (n=3). Histological findings were examined by cresyl violet staining, and DNA fragmentation was examined by specific labeling of 3'-OH DNA ends using the TUNEL technique. In Tg mice, hsp70 was strongly expressed in cortex, caudate putamen, thalamus and whole hippocampus at 4 h, and it was still detectable within the hippocampal CA1 for up to 24 h. However, in nTg mice, hsp70 weakly appeared only CA1 from 4 to 24 h. There was no observable neuronal necrosis in either Tg or nTg by cresyl violet, and DNA fragmentation was not seen in either Tg or nTg. The present study demonstrates that increased CuZn-SOD activity induced or permitted the hsp70 expression after transient bilateral CCA occlusion without any observable cerebral injury. We speculate that upregulation of hsp70 expression may be associated with decreased production of reactive oxygen species in CuZn-SOD Tg mice after a transient cerebral ischemia. (Supported by NS-14543, NS-25372, AG-08938.)

675.19

ASTROCYTE SURVIVAL AND HSP70 HEAT SHOCK PROTEIN INDUCTION FOLLOWING ACIDOSIS AND HEAT SHOCK. P.Narasimhan*, R.A.Swanson, S.Sagar, F.R.Sharp. Department of Neurology, University of Californ at San Francisco and SFVAMC, San Francisco, CA 94121.

Though severe acidosis is animportant mediator of brain infarction, recent evidence suggests that mild acidosis may also protect ischemic cells. The HSP70 heat shock protein is induced by acidosis in cultured cells and in ischemic brain, and is known to protect cells against many types of injury. Therefore, this study determined whether induction of heat shock proteins protects cultured astrocytes against acidosis. Brief exposure of cultured cortical astrocytes to acid (pH5.2 for 40 minutes) or heat shock (45^{0}C for 40 min) markedly induced hsp70 mRNA and HSP70 protein in these cells. Heat shock of the cultured cortical astrocytes completely protected the astrocytes from subsequent heat treatment (45^{0}C for 4h). In contrast, heat shock pretreatment and acid pretreatment sensitized the astrocytes to injury from acidosis 24 h later. Heat shock pretreatment did protect astrocytes against exposure to acid 48h later. These results suggest that induction of other stress proteins and acid shock proteins partially protect the astrocytes against damage produced by high concentrations hydrogen ions.

675.20

ANOXIA/AGLYCEMIA INDUCES mRNA ENCODING THE 70 KDA STRESS PROTEIN, HSP72, IN RAT HIPPOCAMPAL SLICES. Q. Zhou* and T. S. Nowak, Jr. Depts. of Anatomy & Neurobiology and Neurology, University of Tennessee, Memphis, TN 38163

In previous studies we examined changes in the expression of mRNAs and proteins that occur during preparation of hippocampal slices. Under optimal conditions hsp72 was not detected, MAP2 immunoreactivity was comparable to that of normal brain, and Fos and Jun staining were only moderately increased. We have now used slices that meet these criteria to develop an in vitro model that replicates many features of postischemic hsp72 expression observed in vivo. Vibratome slices (400 μm) were prepared from halothane anesthetized Wistar rats and incubated at 34 °C in standard artificial cerebrospinal fluid (ACSF) purged with 95% O_2, 5% CO_2 including 5 mM glucose and 1.5 mM Ca. The anoxic/aglycemic insult was produced by transfer to ACSF lacking glucose, replacing O_2 with N_2 and reducing Ca to 0.2 mM. After insults of 2-6 min slices were returned to normal ACSF for varied intervals up to 4 h. Slices were frozen or fixed in 4% paraformaldehyde and sectioned for in situ hybdridization, immunocytochemistry and histology. Insults of 2-3 min duration resulted in minimal changes in normal MAP2 immunoreactivity during the time intervals examined, while anoxia/aglycemia of 4 min or longer produced rapid loss of MAP2 staining and histologically shrunken neurons. Hsp72 mRNA was detected after reoxygenation intervals of 1 h or longer, with preferential induction in dentate granule cells and CA3 neurons. Preliminary results indicate maximal expression after 3 min in vitro ischemia. These findings demonstrate the potential utility of such preparations for studies of changes in gene expression after ischemia.

676.1

PREVALENCE OF TRAUMATIC BRAIN INJURY IN RUGBY AND LACROSSE PLAYERS. J. Liotta, P. Donovick*. Environmental Neuropsychology Lab., Dept. of Psychology, State Univ. of N.Y. at Binghamton, 13902-6000.

The present study of 59 athletes was done to see the amount of traumatic brain injury (TBI) that they have sustained while playing in their respective sports. An inventory of their sports history was also examined. The athletes currently play women's lacrosse or men's and women's rugby. The testing instrument was the Binghamton Head Injury Questionnaire. Two trends were observed. The first being that rubgy players had a higher incidence of TBI than lacrosse players. The second was that female rugby players had a higher incidence of TBI on avg. than males in both their sports history and in rugby. It was concluded that this may be due to the amount of experience that both sexes have with contact sports. Females may have reported every incident while males only reported the severe cases because they are used to the little blows. Another conclusion was that males have more developed necks and their heads are more protected as a result.

676.2

SINGLE-DAY TESTING IN THE MORRIS WATER MAZE. R.F. Cody, D.O. Maris, L.C. Costa*[1], M.S. Grady. Depts. of Neurological Surgery and Environmental Health[1], Univ. of Washington School of Medicine, Seattle, WA.

The Morris water maze has been widely used to evaluate spatial learning. Usually, sequential trials are carried out over several days to allow subjects time to acquire the task. We were interested in determining whether a series of trials in a single day would retain test specificity and sensitivity and have the benefit of reducing work effort required in analyzing spatial function. In this study, the Morris water maze was used to test spatial learning in naive adult male Sprague-Dawley rats at 7 and 14 days following either sham surgery or experimental traumatic brain injury (fluid percussion injury) (n=8 for each group). Six trials per day were used, for a total of 12 trials per rat. Escape latency, quadrant time, and cumulative platform distance were measured with a video tracking system (VP-116, HVS Image, Hampton, England). Spatial bias was assessed with free swims (probes) on the 5th and 12th trials.

Sham rats demonstrated significantly better performance than injured rats in both probe trials. Cumulative distance measures indicated task acquisition by sham but not injured animals at the end of the first day ($p<.009$ to .03); significance was approached but not obtained in quadrant time and latency measures ($p<.053$ to .147). Comparison of escape latency, quadrant time, and cumulative platform distance indicated definite acquisition of the task by sham rats at the end of the second day of testing ($p<.001$ to .017). This experiment shows that single-day testing in the Morris water maze can be used to assess spatial learning accurately.

676.3

EFFECTS OF SECONDARY INSULT ON TRAUMATIC BRAIN INJURY. D.A. Chorney*, J.S. Soblosky, L.L. Colgin, J.F. Davidson, and M.E. Carey. Dept. of Neurosurgery, LSU Medical Center, New Orleans, LA 70112

Secondary insults (eg. hypotension, hypoxia) potentiate traumatic brain injury. We quantified the effects of traumatic brain injury followed by 10 minutes of hypotension (MABP 50 mm Hg) on rodent sensory/motor behavior. Isoflurane-anesthetized rats were injured in the right sensory/motor cortex by a piston with a 4X8mm elliptical tip which depressed the dura 1mm. Impact speed was 5M/sec. Hypotension was produced by removing 5ml of blood via a jugular cannula. We reinfused the blood after the 10-min hypotensive period. Animals were placed in one of four groups: 1) Brain-injured followed by 10-min. of hypotension; 2) 10-min. hypotension only; 3) brain-injured only; and 4) control. We evaluated motor deficits using four tests: performance traversing a flat narrow beam, the number of footslips on a pegged narrow beam, the number of foot-faults on a grid platform, and a forepaw preference test. The results indicate the animals that received brain injury and were made hypotensive performed significantly poorer on the flat beam and the grid than the normotensive brain-injured rats or the rats subject to hypotension. Experimental brain injury followed by hypotension has a demonstrated deleterious effects on motor behavior, beyond that of brain-injury alone.

676.4

HYPERMETABOLIC RESPONSES TO BRAIN DAMAGE S.Y. Roe, J.K. Relton+, S. Toulmond, E. McGowan* and N.J. Rothwell School of Biological Sciences, Neuroscience Division, 1.124 Stopford Building, University of Manchester, Manchester M13 9PT, UK +Present address Amgen, 1885 33rd Street, Boulder, Colorado 80301, USA

Increases in metabolic rate have been reported in patients with traumatic or ischaemic brain damage, and can compromise neurological recovery and lead to metabolic disturbances. In order to investigate the mechanisms underlying these responses, metabolic rate has been measured after focal ischaemia (middle cerebral artery occlusion, MCAo) or traumatic brain injury (lateral fluid percussion) in the rat. Both insults lead to significant (25-30%) increases in resting oxygen consumption over the 24h period after injury. This hypermetabolism was dependent on activation of the sympathetic nervous system and was associated with reduced food intake and weight loss. Expression of corticotrophin releasing factor (CRF), which has been implicated in metabolic responses to systemic injury and infection, was induced by brain damage. Central (icv) injection of a CRF receptor antagonist, or lipocortin-1, which inhibits CRF release, significantly inhibited the increases in metabolic rate induced by MCAo. These data suggest that the marked metabolic disturbances which result from brain damage share common mechanisms with those seen after systemic injury, and depend on synthesis of CRF.

676.5

PHARMACOLOGICAL ATTENUATION OF PHOTOCHEMICALLY INDUCED APOPTOSIS IN THE RAT BRAIN. A. Kharlamov*, D.M. Armstrong and H. Maney. ASRI, Medical College of Pennsylvania and Hahnemann University, Allegheny Campus, Pittsburgh, PA, 15212.

It has recently been demonstrated that apoptotic cell death characterized by intranucleosomal DNA fragmentation can occur in a mature brain after ischemic damage (Stroke 1993, 24:2002; J Neurochem. 1993, 61:378; NeuroReport 1994, 5:493). We have shown in rats that photothrombotic focal brain injury, induced by the intravenous injection of the photosensitive dye rose bengal and skull irradiation with a beam of focused light, can initiate the apoptotic process in cells in the area surrounding the necrotic core (NeuroReport 1994, 5:2661). Apoptotic cells were identified using the immunocytochemical terminal deoxynucleotidyl transferase (TDT)-mediated dUTP-biotin nick end labeling (TUNEL) technique (ApopTag kit, Oncor). In addition, the area of the infarcted core was measured in brain slices incubated with 2% triphenyltetrazolium chloride for 15 min at 37°C. Further evidence of programmed cell death was obtained using a pharmacological approach and blocking: a) endonucleases by intracerebroventricular injection of aurintricarboxylic acid (ATA 5,10,20 µg/5µl); and b) protein synthesis by injecting cycloheximide subcutaneously (2.5 mg/kg). Both treatments significantly reduced the number of apoptotic cells in the area surrounding the necrotic core, suggesting that the apoptotic process involved de novo synthesis of proteins. In this study we also blocked glutamate receptors by injecting MK-801 intravenously (2.5 mg/kg). MK-801 reduced the size of the photothrombotic lesion and decreased the number of TUNEL-labeled cells. Our results are consistent with the hypothesis that glutamate receptor activation (probably caused by the release of glutamate from the lesion core) may trigger delayed apoptotic cell death. Treatments targeted at halting this process might provide neuroprotection in post-stroke or post-traumatic brain pathologies.

676.6

ASTROCYTE INJURY: THE ROLE OF EXTRACELLULAR CALCIUM B.A. Rzigalinski, S. Liang, and E. F. Ellis*. Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond VA 23298.

Although considerable evidence supports a role for elevated intracellular calcium ($[Ca^{2+}]i$) in neuronal damage associated with traumatic injury, mechanisms which mediate traumatic injury in the astrocyte are less clear. Using an *in vitro* model of cell injury (Ellis et al., FASEB J. A665, 1994), we investigated the role of Ca^{2+} in astrocyte injury. Neonatal rat cortical astrocytes were cultured in silastic-bottom wells. Stretch injury was induced using a Cell Injury Controller. This device delivers a regulated pressure pulse of air to the silastic membrane, resulting in membrane deformation and stretch injury. For these experiments, silastic membranes were subjected to a 50 msec pulse, resulting in a 6.3 mm deformation of the silastic membrane (38% stretch). The extent of cell membrane injury was assessed by uptake of propidium iodide (PI) and release of lactate dehydrogenase (LDH). The highest levels of PI staining were observed in cells injured in Ca^{2+}-free or low Ca^{2+} medium (0-10 µM). Increasing concentrations of extracellular calcium (0.1-2 mM) resulted in decreased PI uptake. Release of LDH was also maximal in Ca^{2+}-free medium, and was also decreased by increasing extracellular Ca^{2+} (0.1-1mM). Thus, the extent of injury was actually reduced by elevated extracellular Ca^{2+}. Neither Ba^{2+}, Mn^{2+}, or Zn^{2+} could substitute for extracellular Ca^{2+}, suggesting that the effect on injury is specific for Ca^{2+}. Experiments conducted with $^{45}Ca^{2+}$ demonstrated that stretch injury induced an influx of extracellular Ca^{2+}. Maximal levels of $[Ca^{2+}]i$ (10 fold that of uninjured controls) were attained by 5 minutes post injury and returned to near basal levels by 2-3 hours post injury. Neither influx of $^{45}Ca^{2+}$ nor the ability of increased extracellular Ca^{2+} to protect cells from stretch injury could be inhibited by nimodipine, CNQX, MK801 or SKF96365. Thapsigargin elevates $[Ca^{2+}]i$ by activation of the capacitative calcium influx pathway, independent of receptor occupation. Pretreatment of astrocytes with 1 µM thapsigargin for 5 minutes prior to injury significantly deceased astrocyte injury. Thus, elevation of $[Ca^{2+}]i$ reduces stretch injury of astrocytes. These results may be critical to evaluation of the role of calcium in mechanical injury to the nervous system. Supported by NIH-NS 27214.

676.7

EPIDERMAL GROWTH FACTOR INDUCES PROSTAGLANDIN H SYNTHASE-2 IN CEREBRAL MICROVASCULAR SMOOTH MUSCLE. G. Rich*, L.J. Prokuski, E.J. Yoder, and S.A. Moore. Dept. of Pathology, University of Iowa, Iowa City, IA 52242-1181

Prostaglandins (PG) are likely to play an important role in regulating normal cerebral vessels and their response to injury. This response often involves proliferation of vascular cells and an inflammatory cell infiltrate. Recently an inducible form of prostaglandin H synthase (PGHS-2) has been described that may be important in inflammatory and proliferative conditions. Thus, we investigated the ability of mouse endothelium (En) and smooth muscle (SM) derived from cerebral microvessels to release PG and express PGHS-2. In routine cultures, both En and SM produce PGE_2 and I_2 and express PGHS-1 and -2 mRNA. Serum deprivation for 48h decreases PG production by > 90% in SM but does not affect En. The drop in SM PG release is accompanied by a loss of PGHS-2 mRNA and protein. Serum and epidermal growth factor (EGF) reinduce PG production in these serum-deprived cultures, while other growth factors, FGF and PDGF, are largely ineffective. Selective PGHS-2 inhibitors, NS-398 and 6-methoxy naphthalene acetic acid, block EGF and serum-induced PG production. Readdition of serum also upregulates PGHS-2 mRNA. Furthermore, EGF and serum induce PGHS-2 protein in immunoprecipitation and immunohistochemistry assays. A specific PGHS-2 antibody labels the nuclear membrane with high intensity in serum and EGF-stimulated cells. While a mechanism for serum and EGF induction of PGHS-2 is not clear, phorbol esters also induce this PGHS suggesting that protein kinase C may be involved. These results indicate that cerebrovascular SM may be a significant source of PG in brain injury. Supported by NS-27914, NS-24621 and NS-09858.

676.8

THE FEASIBILITY OF SPINAL CORD STIMULATION FOR CONTROL OF LOWER EXTREMITIES. V. K. Mushahwar, K. W. Horch and P. R. Burgess*. Dept. of Bioengineering, Univ. of Utah, Salt Lake City, UT 84112

The goal of this project is to assess the feasibility of controlling muscles in the hindlimb of cats by electrically stimulating the spinal cord, ultimately as a method for restoring mobility to paralyzed individuals. Earlier work in our lab has shown that the lumbo-sacral portion of the spinal cord contains regions in which stimulation activates specific muscle groups in the leg in isolation. We refer to these regions as "activation pools." We report here on the activation pools for the quadriceps muscle group.

For the experiments, the lumbo-sacral portion of the spinal cord was exposed. The patella was dislocated and the patellar tendon was transected and attached to a transducer to measure quadriceps force. Activities in the remaining main muscle groups were monitored using a combination of force transducers and EMG electrodes. Electrical stimulation through penetrating needle electrodes was used to map the quadriceps activation pools in the ventral horn of the cord.

The results show that the quadriceps muscle group has two activation pools. The rostral pool spreads from mid L5 to mid L6, while the caudal pool is contained within L7. Current thresholds in the quadriceps pool for force production were as low as 3 μA for 600 μs pulses. Stimulus levels could be increased several fold beyond threshold before activity in other muscle groups was detected. Peak twitch forces as high as 17 N were generated by single point stimulation.

These results indicate that the quadriceps muscle group can be selectively recruited by electrically stimulating activation pools in the lumbo-sacral portion of the spinal cord, and that good control of force can be achieved. If, as expected on the basis of preliminary work we have completed, these results can be extended to the remaining muscle groups in the leg, it would be feasible to utilize spinal cord stimulation for restoring mobility to paralyzed individuals.

MENTAL ILLNESS II

677.1

PERSISTENT SLEEP ABNORMALITIES AND CLINICAL COURSE IN PRIMARY ALCOHOLICS WITH SECONDARY DEPRESSION **CPClark,JCGillin,SGolshan,ADemodena,TLSmith,SDanowski,SLFoote* Mlrwin,MASchuckit Dept. of Psychiatry, UCSD, La Jolla CA 90048.**

23 male primary alcoholics with secondary depression (PASD) were compared retrospectively with 23 age-matched males with alcoholism alone. Polysomnography (PSG) and Hamilton Rating Scale for Depression (HRSD) scores at admission & discharge were analyzed (mixed design ANOVA, BMDP). Subjects were diagnosed by Schuckit's $1^{o}/2^{o}$ classification system & were excluded for alcohol withdrawal, other Axis I diagnoses, recent drug use, recent delirium tremens, serious medical problems, or sleep disorders. All remained medication-free throughout their admission.

HRSD-17 was significantly worse in PASD than in pure alcoholics. HRSD-24, HRSD-17, sleep latency, and % sleep efficiency improved by discharge in both groups. REM duration displayed a trend toward interaction, decreasing slightly in the pure alcoholics as compared to little or no increase in PASD. Delta sleep abnormalities were severe in both groups at admission; like some other sleep abnormalities, they persisted despite clinical improvement.

Studies in primary depression suggest that short REM latency and decreased delta sleep may be persistent trait markers.

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677.2

BINDING TO 5-HT_{1D} SITES IS REDUCED IN PREFRONTAL CORTEX OF ALCOHOLICS. *V. Arango*, V.D. Khait and J.J. Mann.* Dep. of Neuroscience, NYS Psychiatric Institute and the College of Physicians & Surgeons of Columbia University, New York, NY 10032.

Alterations in the serotonergic system have been implicated in suicidal behavior and in chronic alcohol consumption. We sought to determine whether binding to 5-HT_{1D}, a presynaptic serotonin receptor, differed between alcoholics who died by suicide (SA, n=12) or other means (CA, n=12), compared to normal controls (C, n=12) in homogenates from dorsal prefrontal cortex. Each SA and CA were carefully matched to a control based on age, sex and postmortem delay (PMI) and assayed together as a triplet. Demographic variables did not differ between groups, including age (SA=37.5±4.0y [20-61]; CA=39.2±4.3y [19-66]; C=38.3±4.1y [17-66], p=0.96) and PMI (SA=11.3±1.1h; CA=12.3±1.2h; C=13.2±1.1h; p=0.51). The ratio of men to women was 10:2 in each group. P_2 pellets were resuspended in 50mM Tris-HCl buffer (pH 7.7) and preincubated (37°C) for 10 min. The incubation (30 min, 20°C) contained six concentrations of ^{3}H-5HT (0.25 to 4nM) with 100nM 8-OH DPAT and 10μM pargyline. Specific binding was defined by 5μM sumatriptan. The levels of 5-HT, its precursor (TRP) and metabolite (5-HIAA) were determined by HPLC.

Both alcoholic groups had lower B_{max} than controls (CA=60.9±9.1, p=0.027; SA=56.4±4.9, p=0.009, C=79.1±8.0 fmol/mg protein), but K_D did not differ between groups. Neither B_{max} nor K_D correlated with age. B_{max} correlated positively with PMI when all groups were examined together (r=0.37 p=0.03, n=36). K_D did not correlate with PMI in any group. The levels of 5-HT, 5-HIAA and TRP did not differ between groups (p>0.05). B_{max} correlated with 5-HIAA only in the control group (r=0.75, p=0.01). K_D did not correlate with 5-HT, 5-HIAA or TRP. The data suggest that alcoholism is associated with damage to serotonergic nerve terminals independent of the serotonergic deficit related to suicide (MH46745, AA09004).

677.3

INCREASED ABUNDANCE OF MONOAMINE OXIDASE-A CATALYTIC SITES AND ACTIVITY IN BRAINS OF HUMANS WITH HEPATIC ENCEPHALOPATHY D.D. Mousseau[1]*, G.B. Baker[2] and R.F. Butterworth[1] [1]Neurosci. Res. Unit, Hôp. Saint-Luc, Univ. of Montreal, Montreal and [2]Neurochem. Res. Unit, Dept. of Psychiatry, Univ. of Alberta Hospital, Edmonton, Canada.

Hepatic encephalopathy (HE) is a neuropsychiatric complication of chronic liver disease which presents an altered biogenic amine function. Monoamine oxidase (MAO) is the main degradative enzyme in amine metabolism. We investigated the binding parameters of [^{3}H]Ro 41-1049 and [^{3}H]Ro 19-6327, ligands having a high-affinity for the catalytic sites of MAO-A and -B, respectively, in autopsied brain tissue from cirrhotic patients with HE. The binding density of [^{3}H]Ro 41-1049 was increased in both frontal cortex (by ~86%) and cerebellum (by ~223%) of HE patients. An increase in MAO activity in the frontal cortex (by ~47%) and in the cerebellum (by ~145%) was also limited to MAO-A. Except for a slight increase in cerebellar activity, MAO-B parameters (*eg.* specific binding of [^{3}H]Ro 19-6327 and activity) in patient tissue were not significantly different from those in control tissue. Analysis of putative neurotransmitter amines in both regions indicated increased levels of 3,4-dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid, the predominant MAO-mediated metabolites of dopamine and serotonin, respectively. These findings suggest a pathogenic role for the MAO-A isozyme in human HE. (Funded by MRC, Canada)

677.4

THETA SLOWING AS A RELIABLE, EEG-BASED INDICATOR OF 5-HT_2 ANTAGONISM. D.F. Sisson*, D.H. Snyder, J.M. Goldstein. Zeneca Pharmaceuticals Group, Wilmington, DE 19897.

Several putative, antipsychotic compounds were administered to rats *per os* as a suspension in HPMC. EEG was recorded from screw electrodes chronically implanted, bilaterally, in the skull over frontal and parietal cortices. The EEG signal was quantitatively analyzed in the frequency domain by estimating the power spectrum of one minute epochs of EEG before drug administration and at intervals following drug administration. Mobility, a metric related to the mean frequency, and power in the theta band (4.0 - 8.0 Hz) were analyzed as a percent of their values before drug administration. Neither HPMC nor haloperidol changed theta mobility or power. Chlorpromazine, clozapine, olanzapine, risperidone, ritanserin and ZD3638 decreased theta mobility. Clozapine and olanzapine increased theta power. The common factor among the compounds that decreased theta mobility was predicted antagonism of the 5-HT_2 receptor at the administered dose. The similarity of the effect on theta mobility between a selective 5-HT_2 antagonist such as ritanserin and a compound with a much broader spectrum of receptor affinities such as clozapine also suggests that the decrease in theta mobility observed in this study is indicative of 5-HT_2 antagonism. Theta slowing induced by serotonergic antagonism has also been observed in the hippocampus (Vanderwolf, *et al.*, 1990). The increase in theta power induced by clozapine and olanzapine can be attributed to the heavy sedation produced by these compounds. Sedation would decrease the overall tone of the serotonergic system (Trulson & Jacobs, 1979), and thereby augment the decrease in theta mobility induced by 5-HT_2 antagonism.

Trulson, M.E. and Jacobs, B.L. (1979). *Brain Res.*, **163**: 135.
Vanderwolf, C.H., Baker, G.B. and Dickson, C. (1990). *Annals of the New York Academy of Sciences* **600**: 366.

677.5

BIOCHEMICAL PROFILE OF BEFLOXATONE, A SELECTIVE AND REVERSIBLE MAO-A INHIBITOR. O. Curet*, G.Damoiseau, N. Aubin, C. Sauvage, N. Sontag, C. Carter, J. Benavides, B Scatton, Central Nervous System Research Department, Synthelabo Recherche, Rueil-Malmaison, France.

Befloxatone selectively and competitively inhibits MAO-A in different rat and human tissues (brain, duodenum, liver and heart) with Ki's ranging from 1.9 to 3.6 nM for MAO-A and from 270 to 900 nM for MAO-B. Befloxatone does not interact with a large number of receptors, monoamine transporters or other amine oxidases. The inhibition of MAO-A by befloxatone is fully reversible in vitro (dilution method) and ex vivo after a single oral administration. In the rat brain, befloxatone inhibits MAO-A with an ED_{50} of 0.018 mg/kg, po, increases the levels of NA, DA and 5-HT and decreases the levels of their respective ideaminated metabolites. In vivo microdialysis studies show that befloxatone (0.75 mg/kg, ip) increases extracellular levels of NA (cortex), DA (striatum) but not 5-HT (cortex) after acute administration. In rat brain sections and homogenates, [^{3}H]-befloxatone labels a single saturable population of binding sites with a Kd of 1.3 and 1.5 nM, respectively wihich has the pharmacological properties of MAO-A. In vitro (brain) and ex vivo (duodenum), the binding of [^{3}H]-befloxatone is displaced by tyramine. In the rat brain, a striking correlation was observed between in vitro and in vivo labelling of MAO-A by [^{3}H]-befloxatone. In conclusion, these data provide evidence that befloxatone is a potent, reversible, competitive, and selective MAO-A inhibitor. Befloxatone is currently undergoing phase II clinical trials.

677.6

LITHIUM DELAYS ONSET OF OUABAIN-INDUCED EPILEPTIFORM-LIKE RESPONSES IN HIPPOCAMPAL SLICES

R. S. El-Mallakh, A. Schurr, R. S. Payne, R. Li, and R. S. Levy*. Depts of Psychiatry, Anesthesiology, and Biochemistry, University of Louisville School of Medicine, Louisville, KY 40292.

Lithium pretreatment has been shown to: protect dogs from digoxin-induced cardiac arrhythmias, protect differentiated cultured human neuroblastoma cells (SY5Y) from ouabain-induced cardiotoxicity, and prevent ouabain-induced behavioral changes in rats receiving intracerebroventricular ouabain. To further investigate this phenomenon in living neuronal systems we examined the effect of lithium on ouabain-induced epileptiform-like population responses induced by electrical stimulation. Male Sprague-Dawley rats (225-275g) were treated with intraperitoneal lithium (2.5 mEq/kg/d) for 10-21 days, then used as the source for 400 µm thick hippocampal slices. Extracellular recording of electrically evoked population spikes were obtained from the stratum pyramidale of the CA1 region using micropipettes filled with artificial cerebrospinal fluid. Lithium treatment alone did not alter population spike response. Ouabain (3.3 µM) reliably produced epileptiform-like responses. Lithium pretreatment significantly delayed onset of ouabain-induced epileptiform-like activity by at least 15 min compared to untreated animals ($p<0.05$). Our data demonstrates that lithium can attenuate ouabain-induced effects in hippocampal slices. This system may prove useful in the study of the mechanism of lithium action.

677.7

CLOZAPINE SELECTIVELY BLOCKS A SUBSET OF $GABA_A$ RECEPTORS. R.F. Squires* and E. Saederup. Nathan Kline Institute, Orangeburg, NY 10962.

Earlier, we reported that clozapine reverses only half of the inhibitory effect of 1 µM GABA on ^{35}S-TBPS binding to rat brain membranes in vitro, and speculated that this might be due to selective blockade of some, but not all, $GABA_A$ receptor complexes (Neurochem. Res. 16:1099, 1991). To test this possibility we combined clozapine pair-wise with 44 other partial reversers, and found 13 that produced some additive reversal of GABA antagonistic effects. Ro5-4864 and tranylcypromine gave the largest additive effects (EC_{50} values ~ 310 nM and 70 µM; additive reversal ~ 22% and 26%, respectively). These results suggest that clozapine blocks GABA selectively at one set of $GABA_A$ receptors while Ro5-4864 and tranylcypromine block GABA at other sets that are not blocked by clozapine. Clozapine produces both GABA-negative (seizures, paroxysmal EEG) and GABA-positive (sedation, anxiolytic, anticonflict, retardation of electrical kindling, blockade of metrazol-induced increases in cerebellar cyclic-GMP) effects. The GABA-positive effects may be indirect, and due to selective blockade of certain types of GABergic disinhibition: for example clozapine may block one type of $GABA_A$ receptor on an inhibitory GABergic interneuron (e.g. $\alpha1\beta2\gamma_2$), but not another type on an excitatory principal neuron (e.g. $\alpha2\beta3\gamma_2$, Benke et al. J. Biol. Chem. 269:27100, 1994). Selective blockade of certain $GABA_A$ receptor complexes may contribute to clozapine's antipsychotic effect.

677.8

ERP STUDY ON DISSOCIATIVE DISORDERS. E.Kirino, H.Fumimoto, R.Inoue and H.Imai* Department of Psychiatry and Neurology, Juntendou Univ.Sch.of Med., Tokyo 113

Event-related potentials (ERPs) during an auditory "odd-ball" paradigm were studied in six patients with dissociative disorders. The patients showed significant reduction in the amplitudes of P300 during dissociative disorders compared with the levels at remission. The latency of P300 did not change significantly during and after dissociative disorders. In two patients who showed a high incidence of mismatch negativity (MMN) during and after dissociative disorders, the amplitudes of P300 increased at remission to a greater extent than in the other four patients who showed a low incidence of MMN, moreover, there was no significant difference in the level of atorophy in the superior temporal plane (STP) on their brain CT scans compared with that in the age- and sex- matched control group. The finding of the change in the amplitudes of P300 suggests that the mechanism of dissociation, which has been considered to be a defence mechanism of hysteria, can be psychophysiologically evaluated by ERPs. The amplitudes of P300 might be a state- dependent biological marker of dissociative disorders. The low incidence of MMN and atorohy of STP suggest the possibility of trait-dependent cerebral dysfunction or fragility in dissociative disorders.

677.9

KYNURENINE PATHWAY METABOLITES IN CEREBROSPINAL FLUID AND PLASMA OF TOURETTE SYNDROME PATIENTS. P. B. Chappell, G. M. Anderson, W. K. Goodman, L. H. Price, L. M. Hall, D. J. Cohen and J. F. Leckman*, Yale Univ. Sch. of Medicine, New Haven, CT 06510

The tryptophan (TRP) metabolites kynurenine (KYN), kynurenic acid (KA), and quinolinic acid (QA) are neuroactive, having actions at the NMDA glutamate receptor. Durson et al. (1994) have recently reported substantial elevations in plasma KYN in medicated Tourette's (TS) patients. A possible role for KYN pathway metabolites in TS also has been suggested by reports of their motor effects in animals, and by reports of reduced plasma TRP levels in TS (Leckman et al.,1984; Comings,1990) and reduced basal ganglia levels of TRP in TS postmortem brain (Anderson et al.,1992).

In contrast to the report of Durson, plasma KYN was only slightly (6-21%) and not significantly elevated in the TS group (N=23); plasma KYN was highly correlated with plasma ACTH secretion in the TS group. CSF levels of KYN and QA did not differ between the TS and control groups. However, the group mean level of CSF KA was modestly (16%, $p<.02$) increased in the TS group (N=30) compared to the normal control group (N=41).

The small increase in plasma KYN may be consistent with an apparant increased stress responsivity in some TS subjects (Chappell et al.,1994). The increase in CSF KA is intriguing given the previous suggestion that TRP metabolism and glutamatergic functioning may be altered in TS (Anderson et al.,1992).

677.10

SYSTEMATIC CHANGES IN CEREBRAL METABOLIC RATE AFTER SUCCESSFUL BEHAVIORAL TREATMENT OF OBSESSIVE-COMPULSIVE DISORDER (OCD). J.M. Schwartz*, P. Stoessel, L.R. Baxter, K. Martin, & M. Phelps. U.C.L.A. School of Medicine, Los Angeles, CA 90024.

Eighteen drug-free subjects with OCD were studied with PET scans before and after ten weeks of cognitive-behavioral treatment. Twelve patients demonstrated clinically significant improvement during the study period; six did not. Three main findings emerged: (1) responders to treatment showed bilateral decreases in caudate nucleus metabolism, divided by ipsilateral hemisphere metabolism (Cd/hem), compared to non-responders. This finding was more robust on the right ($p=.003$) than the left ($p=.02$). (2) Before treatment there were significant ($p<.002$) correlations of brain activity between the orbital cortex and the caudate nucleus, cingulate gyrus, and the thalamus on the right. After effective cognitive-behavioral treatment these correlations decreased significantly. (3) There was a significant positive rank-order correlation between percentage change in symptom severity rating score before and after treatment and the percentage change in orbital cortex/hem on the left ($p=.003$).

These findings demonstrate systematic changes in brain function in association with successful cognitive-behavioral treatment.

677.11

AUTONOMIC AND STARTLE RESPONDING DURING IMAGERY AND PICTURE VIEWING IN A PATIENT PRE- AND POST-ANTERIOR CAPSULOTOMY TO RELIEVE INTRACTABLE OBSESSIVE- COMPULSIVE DISORDER. S.Y. Musil*, S.A. Martinez, D.J. Drobes, B.N. Cuthbert, W.K. Goodman, and P.J. Lang. Center for the Study of Emotion and Attention, Dept. of Clinical and Health Psychology, Dept. of Psychiatry, Univ. of Florida, Gainesville, Florida 32610.

Anterior capsulotomy, a surgical procedure disrupting fibers connecting prefrontal cortex and thalamus, has been used to treat severe obsessive-compulsive disorder (OCD) when standard clinical interventions have failed. Reports of this procedure typically include extensive pre- and post- surgical evaluations of psychological and neuropsychological functioning. However, to date, no study has investigated changes in physiological reactivity to emotional stimuli which might result from anterior capsulotomy.

We report here on a case of a 33-year-old patient (WW) with a 23-year history of severe OCD which proved refractory to standard treatments, including multiple drug trials, intensive behavior therapy, and ECT. Prior to undergoing anterior capsulotomy via stereotactic radiosurgery techniques, WW participated in several physiological assessment procedures currently in standard use in the Fear and Anxiety Disorders Clinic at UF. Heart rate, skin conductance, corrugator EMG, and eyeblink startle responses were collected during an affective imagery procedure and while the patient viewed a series of affective slides. Prior to surgery, WW showed enhanced startle responding, and increased heart rate, skin conductance, and corrugator EMG, when viewing or imaging fear-related, rather than neutral or pleasant stimuli. These data will be presented and contrasted with post-surgical data.

677.12

CLONING OF NOVEL TRIPLET REPEAT CONTAINING GENES IN RAT FRONTAL CORTEX. M. Ikeda* and M. Nomura Dept . of Physiology, Saitama Med. School, Moroyama, Saitama 350-04, Japan.

The findings that expansion of triplet repeat is responsible for several neuropsychiatric disease has introduced new concepts in human inherited illness. In such diseases including Huntington's disease (HD), spino-cerebellar ataxia type 1 (SCA1), dentatorubral and pallidoluysian atrophy (DRPLA), Machado-Joseph disease (MJD), earlier age at onset, increasing severity of illness are associated with triplet repeat expansion. Marked expansion of triplet repeat may explain the phenomenon of genetic anticipation. Recently, anticipation has been reported in schizophrenia pedigrees suggesting a possible implication of triplet repeat expansion in schizophrenia. To identify novel triplet repeats containing genes associated with neuropsychiatric diseases, PCR was used to isolate cDNA fragments with triplet repeats. Priming with an oligonucleotide 10 CAG repeats and sequences in the phage vector results in several clones with CAG repeats. DNA sequencing and computer data base search to GenBank and EMBL demonstrated that these clones are novel molecules. Northern analysis of these genes is in progress.

MENTAL ILLNESS—DEPRESSION

678.1

LATE ONSET DEPRESSION AND MRI DETERMINED BRAIN VOLUMETRIC MEASURES A Kumar*, D Miller, W Bilker, D Ewbank, D Walsh, S Arnold, G Gottlieb University of Pennsylvania School of Medicine, Philadelphia, PA 19104

The purpose of our study was to examine the relationship between the age of onset of the first episode of major depression and global and regional normalized volumetric measures in elderly subjects with Major Depressive Disorder (MDD). Our sample comprised of 26 subjects (7 M, 19 W, Mean age $\pm$ SD=75.1±6.6) with late onset MDD -- the first episode occurring after age 60. All subjects had Hamilton depression scale scores of $\geq$ 15 and stable comorbid medical disorders with no evidence of clinical dementia or other brain disease. MR scans were performed on a 1.5 Tesla GE signa scanner with head coil (TR=3000, TE=30,80 msec). A segmentation algorithm was used to segment brain parenchyma from CSF and whole brain, hemispheres and frontal and temporal lobes were outlined as previously described (Cowell et al. J Neurosci 1994). Absolute volumes of brain and cerebrospinal fluid (CSF) were normalized using intracranial volume to correct for variations in head size. A multiple linear regression was used to examine the relationship between normalized MRI measures and age of onset of illness while adjusting for the subjects current age. Normalized whole brain CSF and total ventricular volumes showed significant increases with age of onset of illness, (r^2=0.48 p<0.05 and r^2=0.63 p<0.05 respectively), while frontal lobe volumes decreased significantly as onset age increased (r^2=0.19 p<0.05). These data demonstrate that in subjects with late onset depression, global and focal measures of atrophy increase with age of onset of illness. They also suggest that neuroanatomical contributions to the disorder may increase with a later age of onset of the illness.

678.2

INTRACELLULAR CALCIUM SIGNALS IN DEPRESSION MODELLED BY SINGLE-CELL RECORDINGS OF T-LYMPHOCYTES. C. Dumais-Huber and J.B. Aldenhoff*. Central Institute of Mental Health, Cell Physiology Laboratory, J5, P.O. Box 122120, D-68159 Mannheim, Germany.

The aim of the present study was to test the hypothesis of an altered Ca^{2+}-homeostasis in psychiatric disorders and more specifically in depression. To this end, T-lymphocytes served as peripheral model of CNS neurons because of their similarity in receptor types and membrane properties. Thus, this easily accessible cell system provides a mean to delineate cellular events along the signal transduction pathway and to detect possible changes during depression as well as during therapy.

$[Ca^{2+}]_i$-measures were performed by fluorescence photometry in *single* T-lymphocytes of 20 patients with unipolar depression (diagnosed according to the DSM III-R criteria; Hamilton rating scale > 18 at first testing, t0) and of 20 healthy controls matched for age and gender. $[Ca^{2+}]_i$ of T-cells was also assessed after patients had been treated for 8 weeks (t_1) either with Interpersonal Psychotherapy (IPT) (N=13) or else with pharmacotherapy (N=7).

The Ca^{2+}-response of T-lymphocytes to phytohemagglutinin (30 µg/ml PHA) was markedly reduced during depression. Not only was the percentage of PHA-responsive T-cells lower in depressed patients (20% in contrast to 50% in healthy subjects), but the recorded Ca^{2+}-signals were altered: longer onset latency, slower $[Ca^{2+}]_i$-rise, smaller area under the curve, fewer Ca^{2+}-oscillations. Upon clinical recovery, all deviant Ca^{2+}-parameters were found to normalize independently of therapeutical approach. Our results indicate that single-cell recordings make it possible to analyze the dynamics of Ca^{2+}-signals and yield information having clinical implications for the monitoring of cellular functions in depressed patients under therapy. (Supported by the German Research Society and by theVolkswagenwerk Foundation. We thank J. Sulger for laboratory work, M.Fritzsche&P. Gabriel for the supervision of patients).

678.3

Behavioural Despair: A drug screening procedure sensitive to hypothermia. Nadeau,*B. G., Marchant, E.G., Mistlberger, R. E., Alexander, B. K. and Amir, S. Psychology, Simon Fraser Univ., Burnaby, B. C., V5A 1S6.

Rats forced to swim after an initial burst of activity become immobile. The onset of immobility is accelerated on subsequent trials. Immobility has been interpreted as a state of lowered mood or despair which is reduced by administration of antidepressants between trials. Despite false positives behavioural despair has a claim to validity as an animal model of depression. Four experiments were conducted which together challenge this claim. Experiment 1: Twenty-four male Wistar rats (250-350g) were immersed in 27ºC water for 30 min on four occasions. Activity decreased across trials and was correlated (r=-.49) with a diminished loss of core temperature. Experiment 2: Ten rats were immersed in either 15 or 35ºC water on trials1 and 2 (15 min for trial 1; 5 min for 2,3,4). Temperature was reversed for trial 3 and reversed again for trial 4. Activity varied with water temperature. Reversal from 35 ºC to 15 ºC water produced an increase in activity, and was decreased by a reversal from 15 ºC to 35ºC water. Experiment 3: Forty one rats were immersed twice in 15, 20, 22, 25, 30, 35, 37, or 40ºC water (5 or 6 per group). Activity in the second trial was a quadratic function of water temperature (r=.51). Only 25 ,30, and 35ºC water produced significant reductions in activity between trials. Experiment 4: Typical, atypical, and non-antidepressants were administered (IP) to rats (5 or 6 per group) between trial 1 and 2 and the rats were immersed in either 25 ºC or 35 ºC water. There were no differences in activity on trial 2 as a function of water temperature for saline treated rats. Compounds which reduced core temperature (-1ºC or more) increased activity in the second trial over the saline control in 25 ºC water. This was not true at 35ºC. It is concluded that activity in the behavioural despair test reflects behavioural thermoregulation and compounds tested at 25ºC, as in the usual procedure, which produce hypothermia result in increases in activity associated with cooler water.

678.4

IS THE DST A VALID MARKER OF PSYCHIATRIC DISORDERS ?
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In the last ten years the dexamethasone suppression test (DST) was used as one of the diagnostic methods for detecting several psychiatric diseases, such as depression. Although in these cases high percent of DST positivity was found, only little attention has been paid to the role of stress in DST positivity.

In this study 140 healthy young males were investigated in the time of a stressful life event (day of military induction). One mg dexamethasone was given p.o. at 22:00 PM and the next day 8:00 AM a blood sample was taken. Cortisol was measured by competitive protein binding method. The cut off point was 5 µg/dl. All subjects underwent a complete physical and psychiatric check up. Thirty eight subject (24%) had an abnormal DST result.One month later only 4% of the subjects showed DST nonsuppression.The results suggest, that a stress situation alone can cause reactively frequent DST nonsuppression in healthy, young men.

678.5

SEROTONIN (5HT) -1A AND 5HT-2A RECEPTORS IN PREFRONTAL CORTEX (AREA 10) AND HIPPOCAMPUS OF SUICIDE VICTIMS WITH MAJOR DEPRESSION. C.A. STOCKMEIER*, G.E. DILLEY, L.A. SHAPIRO, J.C. OVERHOLSER, P.A. THOMPSON, AND H.Y. MELTZER. Departments of Psychiatry and Psychology, Case Western Reserve University, Cleveland, OH 44106.

Several studies in the past decade have yielded conflicting results on altered numbers of 5HT receptors in prefrontal cortex and hippocampus in depression and suicide. In the present study, we examined the hypothesis that 5HT-1A and 5HT-2A receptors were increased in suicide victims with major depression. Samples of right prefrontal cortex (area 10) and hippocampus were collected at autopsy from 20 suicide victims and 19 control subjects. Retrospective psychiatric assessments were conducted for all suicide victims and most of the age-matched control subjects. [^{3}H]8-OH-DPAT and [^{3}H]ketanserin binding to 5HT-1A and 5HT-2A receptors, respectively, was measured using quantitative receptor autoradiography. Neither age nor postmortem interval significantly affected these receptor measures. In either brain region, there were no significant differences in radioligand binding between all suicide victims and control subjects, or between a subset of suicide victims (N=11) with major depression (and no psychoactive substance use disorder) and control subjects. In the latter group of suicide victims, no antidepressant or antipsychotic drugs were detected postmortem, and only 2 had recent prescriptions for an antidepressant drug. In conclusion, neither 5HT-1A nor 5HT-2A receptors were altered in prefrontal cortex (area 10) or hippocampus of suicide victims with a history of major depression. Supported by PHS Grants MH45488 and MH41684.

678.6

ANTIDEPRESSANT/ANTIPANIC DRUGS MODULATE GABA$_A$ RECEPTOR GENE EXPRESSION. A.N. Bateson[1,3]*, V.A.-M.I. Tanay[1], A.J. Greenshaw[2,3] and G.B. Baker[2,3]. Departments of [1]Pharmacology and [2]Psychiatry, and [3]Division of Neuroscience, Faculty of Medicine, University of Alberta, Edmonton, Canada T6G 2H7.

Pharmacotherapy of panic disorder can be achieved with a number of classes of drugs that apparently have differing primary sites of action. These include monoamine oxidase inhibitors (MAOs), tricyclic antidepressants (TCAs) that inhibit noradrenergic and serotonergic uptake, and benzodiazepines (BZs) that potentiate GABA$_A$ receptor function. Certain MAOs and TCAs have been shown to have secondary sites of action that result in increased brain GABA levels. It is possible therefore that GABAergic transmission underlies the etiology and/or pharmacotherapy of panic disorder. Previous studies have indicated that long-term activation of GABA$_A$ receptors results in specific changes in GABA$_A$ receptor gene expression. We have chronically exposed rats to representative drugs from each of the therapeutic classes that are used in the treatment of panic disorder to determine whether they produce similar changes in GABA$_A$ receptor gene expression. Drugs were delivered via subcutaneously-implanted osmotic minipumps and specific GABA$_A$ receptor subunit mRNA levels determined using a multiprobe solution hybridisation assay. We have found that BZs and TCAs alter specific GABA$_A$ receptor mRNA levels suggesting a novel action of these drugs that may play a role in their therapeutic profile of panic disorder.
Funded by the Alberta Mental Health Research Fund and the Canadian Psychiatric Research Foundation.

678.7

LIMBIC TRH IS DIFFERENTLY AFFECTED BY ANTIDEPRESSANTS (AD) vs SEIZURES. R.L. Lloyd*, A.E. Pekary, M. Chilingar, A. Sattin. Psychiatry, Medicine (Endocrinology) & Research Services, W. Los Angeles VA Medical Center & UCLA, Los Angeles, CA 90073.

Electroconvulsive seizures (ECS) are known to trigger bursts of synthesis of preproTRH in neuronal nuclei of several limbic regions, resulting in long-lasting increases of TRH (pGlu-His-Pro-NH_2) and its immediate precursors including TRH-Gly. In the seizure model, the magnitude of the TRH-Gly *increase* is significantly related to the reduced immobility (AD effect) in the forced-swim test (FST) (Ann NY Acad Sci, 739:135, 1994). Bupropion is an effective AD drug and MIF-1 (Pro-Leu-Gly-NH_2) is, like TRH, another endogenous neuro-tripeptide. A clinical AD effect of MIF-1 has been reported (J Affect Disord, 31:227, 1944). We tested both agents in the conventional Porsolt FST (buprop, 15 and MIF-1, 0.01, 0.1 and 1.0 mg/kg ip), applying the method used in the ECS study to relate the swim score of each rat to the peptide levels (by RIA) in extracts of brain regions. Others had previously shown that both drugs reduced immobility in the FST. We confirmed this using young male Wistars from Simonsen (Gilroy, CA). Neither drug altered mean level of either TRH or TRH-Gly in any region, but in amygdala/entorhinal cortex, the reduced immobility by both drugs, was directly related to *decreases* in the peptide precursors of TRH as measured by TRH-Gly. MIF-1 (but not bupropion) also showed this in striatum. The MIF-1 effect was dose-related. Such effects were not seen in anterior or pyriform cortices or hippocampus. Unlike ECS, these AD agents might act mainly through alteration of TRH processing. Generalization to other AD drugs and pharmacological specificity remain to be determined. Supported by VA Research Service.

678.8

REDUCED TRH IN LIMBIC FOREBRAIN OF A PRIMATE IS RELATED TO DEPRESSIVE BEHAVIORS. A. Kling*, A.E. Pekary, G. Bramner, R.L. Lloyd, M.E. McGuire, M.J. Raleigh, A. Sattin. Psychiatry & Medical (Endo.) Svcs., Sepulveda & W. LA VA's & UCLA, Los Angeles, CA 91343.

Last year we presented the behavioral data from 4 adolescent female Vervets all of which were craniotomized and bilaterally olfactory bulbectomized (OBX)(2) or control handling (2). Depressive behaviors in the OBX's were shown by reduced active grooming and social approach, and increased social withdrawal. Most of these defects were reversed following electroconvulsive therapy, but during subsequent months the OBX's are believed to have relapsed since one delivered, then neglected its infant. 11-12 mo post-surgical, the 3 survivors (1 OBX died) were sacrificed and 13 anterior limbic, cortical and caudate regions were dissected from each hemisphere for extraction and analysis of TRH. Compared with the 2 controls which were similar, TRH in the OBX was moderately or severely reduced in 11 regions. For selected regions, co-plotting these values with OBX-affected behavior scores gave predictable linear plots implicating TRH deficiency in depressive behaviors. Amygdalar TRH was not related to agression. Support: VA Research Service.

678.9

MEASUREMENT OF HUMAN LYMPHOCYTE G-PROTEIN mRNA LEVELS IN DEPRESSED PATIENTS. T. Ishikane*, J. Kusumi, T. Akitaya*, M. Mitsuhashi* and T. Koyama. Dept. Psychiat., Hokkaido Univ. Sch. of Med., Sapporo 060, Japan. *Hitachi Chemical Co.,ltd, Japan. *Hitachi Chemical Research Center, USA.

Increasing evidence implicates abnormalities in the signal-transducing G protein in the pathophysiology of affective disorder. We designed an easy measuring system of human lymphocyte G protein mRNA in order to study G protein-coupled second messenger systems in depressed patient. After synthesizing cDNA library from lymphocyte mRNA, the G protein cDNA was amplified using polymerase chain reaction (PCR) with biotin-labeled specific primers to all subtypes of G protein. PCR product was hybridized to each G protein subtype (Gs, Gi-1, Gi-2, Gi-3 and Go) specific probe which was immobilized onto microtitre plate. The biotin-labeled G protein subtype cDNA was reacted with alkaline phosphatase-streptavidin conjugate, and then substrate solution was added and color development detected by microplate reader. Using this method, we measured each subtype of G protein mRNA in lymphocyte from 15 drug-free depressed patients (eleven major depression with melancholia, 4 major depression without melancholia) and 15 normal controls. There was no significant difference of any subtypes of G protein mRNA levels between the patient group and controls.

678.10

EFFECT OF PROLONGED TREATMENT WITH ANTIDEPRESSANT DRUGS ON THE LEVEL OF mRNA CODING FOR D-1 AND D-2 DOPAMINERGIC RECEPTORS IN THE RAT BRAIN. M.Dziedzicka-Wasylewska, R.Rogoż, V.Klimek, A.Pilc*
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The effect of prolonged administration of imipramine (IMI), citalopram (CIT) and (+)oxaprotiline (OXA) on the level of mRNA coding for dopaminergic (DA) receptors D-1 and D-2 in the rat brain was studied. Drugs (10 mg/kg) were administered twice daily, p.o., for 14 days. Animals were decapitated 2 or 72 h after the last dose of the drug. The level of mRNA coding for D-1 and D-2 DA receptors was measured in coronal sections of the rat brain through the nucleus caudatus (NC) and nucleus accumbens septi (NAS) by *in situ* hybridization, using commercially available probes (DuPontNEN), labelled with [^{35}S]dATP. Prolonged treatment with IMI (but not CIT or OXA) decreased the level of mRNA coding for D-1 DA receptor, to the same extent in the NC and in the NAS, both shell and core. This effect corresponds well with the decrease in the density of receptors labelled with ^{3}H-SCH23390. On the other hand, the level of mRNA coding for D-2 DA receptor was significantly up-regulated following chronic administration of IMI and CIT (but not OXA) and the effect was more pronounced in the NAS-shell than in the NAS-core and NC. Since there was no change in the binding parameters of ^{3}H-spiperone after prolonged administration of antidepressant drugs (ADs) and behavioral studies undoubtly indicated the potentiation of DAergic transmission by ADs, the present results might indicate the different level of regulation, namely the mRNA coding for DA receptors. (Supp. by KBN grant #6P20708206, Poland).

678.11

PLA_2 ACTIVITY IS INCREASED IN BLOOD OF PATIENTS WITH SCHIZOPHRENIA AND BIPOLAR AFFECTIVE DISORDER. B.M. Ross*,C. Hudson, J.C. Erlich, J.J. Warsh and S.J. Kish, Clarke Institute of Psychiatry, Toronto, Ontario, Canada.

Magnetic resonance spectroscopy studies have suggested above normal turnover of membrane phospholipids in brain of patients with schizophrenia. One possible explanation for these findings is an increase in the activity of the phospholipid catabolizing enzyme phospholipase A_2 (PLA_2). As a test of this hypothesis we compared the activity of PLA_2 in serum obtained from 26 individuals with schizophrenia and 22 individuals having bipolar affective disorder, with that in 33 neurologically normal age matched controls. PLA_2 activity was measured either fluorometrically (Gattaz et al., *Biol. Psychiat.* 28, 495), or radiometrically using whole bacterial membranes as substrate (Albers et al., *Pharmacopsychiat.* 26, 94). When assayed fluorometrically, serum PLA_2 activity in individuals with schizophrenia was significantly increased by 47% compared with control activity ($P<0.0001$). In addition, a smaller 26% increase was observed in subjects with bipolar affective disorder ($P<0.01$). However, when the same serum samples were assayed for PLA_2 activity using the radiometric procedure there was no significant difference between the three groups of subjects (one-way ANOVA; $P>0.95$). Furthermore, there was no correlation between the activity as measured by each assay ($r=0.073$). Thus, our data support the hypothesis that increased phospholipid turnover in schizophrenia could be explained by accelerated phospholipid breakdown mediated by PLA_2. However, the different results obtained using the two different PLA_2 assays, indicate that PLA_2 alterations in schizophrenia are probably limited to specific PLA_2 subtypes. Supported by the Ontario Mental Health Foundation.

678.12

WHOLE BODY ACUPUNCTURE ADDITIONALLY APPLIED TO A MIANSERIN MEDICATION IN THE TREATMENT OF DEPRESSION. Ch. Wolf, S. Bech, T. Neuerer, J. Röschke*, Dept. of Psychiatry, University of Mainz, Germany

The aim of the study was to explore the efficacy of additionally applied acupuncture in the pharmacological treatment of depression with mianserin. For this purpose we randomly included inpatients suffering from a major depressive episode (DSM-III-R) into three different treatment groups: (1) verum acupuncture, (2) placebo acupuncture, (3) controls. Mianserin treatment (120 mg/die) was applied to each of the patients, verum/placebo acupuncture was applied single blind. Psychopathology was rated by independent judges (not informed about acupuncture conditions) at the beginning of the study and twice a week during the treatment period of four weeks (BRMS, CGI, GAS). Whole body acupuncture was applied three times a week at 13 specific or nonspecific point, respectively. 24 patients experienced acupuncture treatment (groups (1) and (2)), 8 patients experienced antidepressant monotherapy. Severity of illness was rated by 21 item Hamilton depression scale (mean HAMD = 29 for acupuncture groups, mean HAMD = 24 for controls). At the end of the study patients with acupuncture experienced an average improvement on the GAS scale of 15 points compared to 9 points under control condition. CGI severity score improved for 1.2 points versus 0.3 points in the control group. BRMS scores were improved for 8 points in case of acupuncture versus 6 points in controls. Taken together, the preliminary results of our study point to the view that acupuncture additionally applied to antidepressant therapy might improve the course of the disease better than pharmacological treatment alone. However, at the time given it is an open question whether these effects should be considered as a placebo effect. A more detailed analysis performed when the study has been finished and separating placebo and verum acupuncture conditions will contribute to this fundamental question.

678.13

AN ANIMAL MODEL OF THERAPEUTIC vs NONTHERAPEUTIC SEIZURES A. Sattin*, A.E Pekary, R.L. Lloyd. Psychiatry Svc., Sepulveda VA, Medicine Svc., W. LA VA & UCLA, Los Angeles, 91343.

In electroconvulsive therapy (ECT, ECS), the grand mal (GM) seizure is necessary but not sufficient for antidepressant (AD) effect (Review: HA Sackeim, Psychopharm Bull 30(3):281,1994). Male Wistar rats were tested for the threshold (Thld) elevation usually seen during clinical ECT, since endogenous TRH (which is increased by seizures) is also a known anticonvulsant. Four groups were given sham only, Thld tests only (x2), and two schedules of ECS. There were three daily ECS, one group "flat" at 9.43 millicoulombs (mC) and one "ramped" at 9.43, 23.5 and 33.mC (unipolar square pulses). Thld tests done a day before were repeated a day after the three daily ECS in1mC increments from 1mC, ending in GM seizure (about 5mC). This 5 day sequence of (corneal) stimuli was bracketed by the forced-swim test of AD effect, then sacrifice. Neither ECS group increased Thld. Both ECS groups reduced swim immobility (AD effect), but the Thld only group did not, despite two GM seizures. In all three groups experiencing seizures, TRH and precursors (TRH-Gly) were increased in 4 limbic & cortical regions, but these peptides were higher in the flat than either Thld or ramp which were similar. As in humans, GM seizures at Thld levels did not show the AD effect. The inverted U effect of current dose on limbic peptides might be explained as follows (and see our data on AD effects of endogenous TRH (Ann NY Acad Sci, 739:135, 1994): Thld doses of current might induce synthesis and processing to TRH and precursors. A supra-Thld dose (flat) might augment synthesis and release, initiating the AD effect, while a further current increase (ramp) enhances processing and degradation of TRH. Support: VA Rsrch. Svc.

678.14

Effects of electro-convulsive shock (ECS) on serotonergic and cholinergic responses in CA1 hippocampal pyramidal neurons of the rat.

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ECS is an effective antidepressant (AD) treatment for severely depressed patients and is often used when AD drug treatments have failed. The ECS-evoked changes in the brain that are instrumental in the AD effect of this treatment have not yet been uncovered. Serotonergic and cholinergic neurotransmission are thought to be involved in the pathophysiology of depression and the mechanism of action of AD drugs. Repeated ECS was demonstrated to increase *in vivo* postsynaptic $5\text{-}HT_{1A}$ mediated responses in rats (De Montigny, 1984). We examined *ex vivo* the effects of ECS on postsynaptic 5-HT and ACh receptor-mediated responses using single electrode current- and voltage-clamp techniques (M_3 and $5\text{-}HT_{1A}$ responses) in hippocampal slices. Extracellular recordings were performed to measure the ACh-induced M_1-mediated reduction of the fEPSP. Rats received 7 convulsive shocks (80 V) every 48 hrs resulting in a total treatment time of 2 weeks. Control groups were given either 7 subconvulsive shocks (20 V) or only one convulsive shock (80 V). All shocks were applied transcranially under light ethrane anaesthesia through subcutaneous electrodes placed at the temples. In the intracellular experiments no clear treatment effects were observed on resting membrane potential, cell input resistance and RC-time. 5-HT-induced outward currents and the concomitant decrease in cell input resistance were similar for all treatment groups. The ACh-induced M_3-mediated depolarization and the subsequent desensitization of this response were also not affected. Extracellular experiments revealed no changes in stimulation currents needed to elicit fEPSP's with a 2 mV amplitude nor changes in the potency and/or efficacy of ACh to reduce this signal. From these results we can conclude that there is no *in vitro* (*ex vivo*) correlate of the ECS-induced increase in the *in vivo* postsynaptic response to 5-HT. Moreover, ECS does not alter M_1 and M_3 mediated cholinergic neurotransmission in the hippocampus of the rat.

678.15

ALTERED LEVELS OF Gαs PROTEIN AND mRNA LEVELS IN POST-MORTEM BRAIN TISSUE IN BIPOLAR AFFECTIVE DISORDER F. Fang, S. Lu, G. Chen, T. Hyde, J. Kleinman, W. Z. Potter, and H. K. Manji*. Dept. of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI 48201, Sec. Clin. Pharmacol. and Clinical Brain Disorders Branch, NIMH, NIH, Bethesda, MD 20892.

Bipolar Affective disorder (BD) is a common, severe, chronic and life-threatening disease. Despite a well established genetic diathesis and extensive research, the biochemical abnormalities underlying the pathophysiology of BD remain unknown. In recent years, studies from independent laboratories have demonstrated elevations in the levels of Gαs in platelets and leukocytes from BD patients, effects which are independent of treatment or mood state. It remains unclear if these are simply peripheral cell abnormalities, or are also observed in the human CNS. We have therefore undertaken a study of Gαs protein and mRNA levels in postmortem brain tissue from a group of BD patients, a group of non-BD subjects who committed suicide, and age- and sex-matched controls. Using selective antibodies, we have found a significant elevation in the levels of both the long (BD: 1649±289; CON: 1321±347) and short (BD: 1394±481; CON: 997±266) form of Gαs in the occipital cortex, but not the frontal cortex. We have also used an RNase protection assay (with a 351 bp fragment of Gαs cDNA as a template) to quantitate the levels of Gαs mRNA levels. 20 µg of total RNA were hybridized with 1.2×10^4 cpm of Gαs riboprobe and standardized to hybridization with 3×10^3 cpm of β-actin riboprobe. We did not observe any differences in the levels of Gαs mRNA levels in the frontal cortex, but observed near-significant decreases in the levels of Gαs (normalized to β-actin) in the occipital cortices of BD subjects (.52±.31) compared to either control subjects (.82±.28; p=.06) or suicide (.84±.23; p=.05) subjects. These findings add to the growing body of evidence implicating complex alterations of the signal transducting G proteins in the pathophysiology of BD, and as targets for therapeutic interventions.

678.16

CEREBRAL GLUCOSE METABOLISM IN LATE ONSET DEPRESSION WITHOUT COGNITIVE IMPAIRMENT M.J. Mentis*, J. Krasuski, P. Pietrini, A Polles, G.E. Alexander, J Szczepanik, D. Murphy, C Grady Lab. Neurosci., Natl. Inst. on Aging, NIH, Bethesda, MD 20892.

Among depressed patients, various clinical and treatment-response subgroups have been reported to have different cerebral functional abnormalities. To evaluate regional glucose cerebral metabolic rates (rCMRglc) in older subjects with first episode major depression (MD) but without cognitive impairment, we compared 11 depressed females (59.1±12 yr., Mini-mental state (MMS) 29.3±0.8,) with 12 age- and sex-matched healthy controls (HC) (62.7±6.2 yr., 10 Female 2 Male, MMS 29.5±0.7) using high resolution positron emission tomography (PET) with 18-fluorodeoxyglucose. MD was diagnosed using Research Diagnositic Criteria and a Hamilton Rating Scale for Depression score (HRSD) greater than 15 (MD group HRSD 22.5±7, HC 0.8±1.5). All scans were performed off drugs. "Secondary" depression was excluded and no subject had medical or cognitive impairment. Each scan was warped into a common three dimensional space (Talairach), then voxel-by-voxel between-group t-tests were performed using the Statistical Parametric Mapping (SPM) program. Compared to HC, the MD group had significantly increased rCMRglc ($p<0.01$) in right medial temporal (amygdala), lateral temporal, orbitofrontal and lateral prefrontal regions, and decreased ($p<0.01$) rCMRglc in both thalamic nuclei, left > right. Limbic and prefrontal importance in depression is supported. Late onset of depression and lack of cognitive impairment may represent a distinct depressive subtype reflected in the laterality and increased rather than decreased metabolic abnormalities.

678.17

A RAT MODEL FOR MANIA: LITHIUM PREVENTS OUABAIN-INDUCED PERSISTENT HYPERACTIVITY. R.Li*, G.Bao, R.S.Levy and R.S.El-Mallakh. Department of Psychiatry and Behavioral Sciences, and Department of Medicine, University of Louisville School of Medicine, Louisville, KY 40292.

We have previously shown that a single dose of intraventrically (ICV) administrated ouabain alters rat locomotor activity (dose-related increase or decrease). In our ongoing efforts to properly model mania, we have recently investigated the effect of chronic ICV administration of ouabain on animal locomotor activity and the action of lithium on these ouabain-induced behavioral changes. Rats were implanted with the ALZET osmotic mini pump filled with ouabain (10^{-3}M) or artificial cerebrospinal fluid (aCSF) at day 0. Pump continuously delivered ouabain or aCSF into left cerebral ventricle for 2-3 weeks. Lithium (2.5mEq/kg/day) or vehicle injections were initiated either one week before or one week after ouabain pump implantation and continued for 4 or 3 weeks, respectively. Locomotor activities of animal were tested by an activity monitor at days 0, 7, 14, 21, 28 and 35. Our results indicate that chronic ICV ouabain infusion induced a significant increase in horizontal activity, total distance, and number of movements ($P<0.05$). This hyperactivity persisted for the entire period of ouabain administration. Lithium pre-treatment prevented ouabain-induced hyperactivity, and lithium post-treatment (one week after ouabain administration) eliminated the hyperactivity but not significantly different from control animals. Our study suggests that a dysfunction of sodium, potassium ATPase may be an important mechanism for manic disorder.

678.18

EFFECTS OF OUABAIN ON TRANSMEMBRANE POTENTIAL OF LYMPHOBLASTS FROM MANIC-DEPRESSIVE AND NORMAL INDIVIDUALS

Lisa Fox, Rif S. El-Mallakh*, Christopher A. Worth, Rena Li, Stephen C. Peiper,. Depts of Psychiatry and Pathology, Univ. of Louisville School of Medicine, Louisville, KY 40292

We have previously noted that lymphocyte transmembrane potential (TMP) is hyperpolarized during mania compared with both euthymic (normal state) bipolars, and normal controls. Since the sodium pump, which is important in determining TMP, has been reported to have decreased activity in manic bipolar subjects, we investigated the effect of acute (3 hr) and chronic (4d) ouabain treatment on TMP of lymphoblasts derived from bipolar individuals (n=5) and normal controls (n=5). Relative TMP was quantified utilizing a flow cytometer and argon laser excitation of the cationic dye D1OC(6), before and after depolarization with gramicidin. Ouabain concentrations of greater than10^{-7} M caused severe cellular disruption in all cell lines. In patient and normal cell lines, respectively, TMP was reduced by 8.7% and 6.1% after 3 hr at 10^{-8} M ouabain, (ns); and by 14.58% and 14.66% after 4 days (ns). The data suggest cell lines from bipolar patients do not differ from normals in their response to ouabain, and sodium pump inhibition is not likely to be involved in producing the lymphocyte hyperpolarization seen in manic bipolar patients.

678.19

BIOCHEMICAL STUDIES OF YM-35992: A NOVEL SELECTIVE SEROTONIN RE-UPTAKE INHIBITOR. T.Yamaguchi*, K.Hatanaka, T.Nomura, H.Takeuchi, M.Fujii, S.Yatsugi. Yamanouchi Institute for Drug Discovery Research, 21 Miyukigaoka, Tsukuba 305 Japan.

YM-35992,(-)-(S)-2-[[(7-fluoro-4-indanyl)oxy]methyl]morpholine monohydrochloride, has dual mode of action, selective 5-HT re-uptake inhibition (SSRI) with 5-HT2 antagonistic activity in vitro and in vivo. Recent microdialysis studies on SSRIs demonstrated that chronic administration with SSRIs increases extracellular 5-HT concentration in the rat frontal cortex but not single administration of those. In the present study, we examined the effect of YM-35992 on 5-HT release in the rat frontal cortex using microdialysis technique comparing with representative SSRI, citalopram. Single treatment with YM-35992 markedly increased the extracellular 5-HT concentration in the rat frontal cortex. YM-35992 at a dose of 30 mg/kg(i.p.) gave more than 6-fold increase in the 5-HT level, while the increase induced by 10 mg/kg (i.p.) of citalopram which corresponds to 30 mg/kg of YM-35992 in terms of in vivo uptake-inhibiting activity was less than 3-fold. The result suggests the early onset of action of YM-35992 clinically. We also examined the changes in 5-HT1A receptors in the hippocampus after long-term treatment with YM-35992 and amitriptyline, a representative tricyclic antidepressant(TCA). Repeated treatment with YM-35992 for 14 days produced significant increase in [^{3}H]8-OH-DPAT binding in the hippocampus. This result indicates that YM-35992, similar to TCAs, could produce 5-HT1A supersensitivity in the rat hippocampus. Although further investigations should be needed, these effects of YM-35992 suggests its potent clinical efficacy.

678.20

THYROID AUTOANTIBODIES IN PATIENTS WITH MOOD AND ANXIETY DISORDERS D.L. Musselman, M.R. Porter, P.T. Ninan, B.T. Knight, R.W. Bonsall, M.S. Emery, Z.N. Stowe, E.J. Hauenstein, K.R.R. Krishnan, J.R. Davidson, and C.B. Nemeroff*. Dept. Psychiat. & Behav. Sci., Emory Univ. Sch. Med., Atlanta, GA 30322.

Previous studies indicate relatively high prevalence rates of anti-thyroid antibodies in depressed patients compared to normal controls. These studies measured titers of these antibodies using generally out-dated, indirect measures, e.g. red blood cell hemagglutination assays. The present study used sensitive methods to quantify the concentrations of anti-thyroglobulin (anti-TG) and antithyroid peroxidase (anti-TPO) microsomal thyroid antibodies in patients with anxiety, depressive, mixed anxiety/depression, post-partum mood disorders, and normal controls. Medically healthy individuals (n=178), without any known history of thyroid disease were studied. Patients with anxiety disorders (n=29), depressive disorders (n=43), comorbid depressive and anxiety disorders (n=22), post-partum depression (n=36), and post-partum women without a psychiatric diagnosis (n=14) were compared to a normal population (n=36) who had no past or present psychiatric disorders. Serum concentrations of T_3, T_4, and TSH were measured by standard techniques; anti-TG and anti-TPO antibodies were determined using the Nichols Autoantibodies Chemiluminescence Assay.

The prevalence of anti-TPO antibodies was greater in women with major depression, anxiety disorder, or comorbid depression and anxiety compared to a normal control population; this finding was particularly striking in women under 40 with comorbid depressive and anxiety disorders. No differences between depressed and non-depressed post-partum patients were detected. Serum TSH concentrations in patients with mood and anxiety disorders were significantly correlated with serum concentrations of anti-TG and anti-TPO. Further studies of HPT axis function in psychiatric patients are warranted. (Supported by NIMH MH-51761.)

NEUROTOXINS III

679.1

INTERACTION OF TWO POLYANIONIC DRUGS (SURAMIN AND PENTOSAN POLYSULFATE) WITH NGF RECEPTORS ON DORSAL ROOT GANGLION NEURONS. C. P. Schumacher*J. S. Gill and A. J. Windebank. Molecular Neuroscience Program, Mayo Foundation, Rochester, MN 55905.

Pentosan polysulfate (PPS), a polyanionic mucopolysaccharide, has been shown to exert inhibitory effects on HIV-1 replication and inhibit paracrine activity of heparin-binding growth factors secreted by a variety of malignant cell lines. Suramin, another polysulfated chemotherapeutic agent has been extensively studied in our lab as a neurotoxic agent. In this study, PPS (100 µg/ml) was found to induce significant cell death in PC12 cells by 48 hours (84% cell viability), but not to the extent that suramin (600 µM) caused cell death (56% cell viability). Of the viable PC12 cells exposed to PPS, we observed limited neurite outgrowth as compared to cells exposed to NGF (10 ng/ml) or suramin (300 µM). Western blot analysis revealed tyrosine phosphorylation of the TrkA receptor (gp 140) in cells exposed to PPS for 10 minutes and 1 hour. The level of phosphorylation was less than that induced by NGF or suramin. In DRG explants, PPS was also found to cause an inhibition of neurite outgrowth in a dose-dependent manner. The level of inhibition was similar to that in suramin treated DRG. Ultrastructural studies were performed on DRG exposed to suramin or PPS for 2, 4 and 6 days. Lamellar inclusion bodies (IB) were consistently observed in suramin treated, but not PPS treated, DRG.

Conclusion. PPS mimics the effect of suramin at the NGF receptor; at low concentrations, it is an agonist and, at higher concentrations, a competitive antagonist. PPS does not, however, cause accumulation of IB which is, therefore, independent of the receptor effects. PPS will be useful in dissecting out suramin's chemotherapeutic activity from its neurotoxic action on DRG (supported by NS 29769).

679.2

FACTORS WHICH MAY HELP EXPLAIN THE AGE-RELATED SENSITIVITY OF RATS TO THE INSECTICIDE CHLORPYRIFOS. S. R. Mortensen, M. J. Hooper, and S. Padilla*. Health Effects Res. Lab., Neurotox. Div., US EPA, R.T.P., NC 27711.

Young animals exhibit an increased susceptibility to the lethal effects of cholinesterase (ChE)-inhibiting insecticides including chlorpyrifos (CPF), although the mechanism(s) for such sensitivity is unknown. Since previous studies in our laboratory indicate that young rat brain acetylcholinesterase (AChE) is not intrinsically more sensitive to CPFoxon (active metabolite), we decided to characterize the blood ChEs of young (PND 4, $n \geq 3$) and adult (≥90 day, $n \geq 3$) male Long Evans rats. Total plasma ChE activity from untreated animals was 60% AChE/40% BChE in PND 4 plasma and 50% AChE/50% BChE for adult plasma. IC_{50}s (30 min at 26°C) were defined concomitantly for PND 4 and adult plasma AChE using CPFoxon. Plasma IC_{50}s showed age-related differences: PND 4 = 5.5 nM and adult = 44 nM. (The brain IC_{50}s were virtually identical [4.9 nM] at both ages.) IC_{50} values may be overestimated when Ca^{++}-dependent hydrolases are not taken into consideration. Therefore, IC_{50}s were also determined in the presence of 1 mM EGTA showing a reduced, but still significant, age-related sensitivity to CPFoxon: PND 4 = 4.4 nM and adult = 12.7 nM. In summary, the AChE of young and adult brain are similar regarding their *in vitro* sensitivity to CPFoxon. There is, however, a significant age-related difference (with and without EGTA) in the sensitivity of plasma AChE to CPFoxon, which partially explains the increased sensitivity of young animals to the anticholinesterase insecticide chlorpyrifos.

679.3

THE EFFECTS OF IBOGAINE ON CLINICAL PATHOLOGY, NEUROPATHOLOGY AND NERVE CONDUCTION VELOCITY IN RABBITS. **G.J. Schaefer*, R.G. York, C.D. King, S.M. Miller, L.J. Beuving, W.F. Jackson, J.M. Huebner, and J.B. Terrill, International Research and Development Corporation, Mattawan, MI 49071, Western Michigan Univ., Kalamazoo, MI 49008 and the National Institute on Drug Abuse, Rockville, MD 20857.**

Ibogaine, a drug proposed for the treatment of opiate and psychomotor stimulant abuse in humans, is reported to cause degeneration of cerebellar Purkinje cells in animals. To determine if the drug is peripherally neurotoxic, adult male New Zealand White rabbits (n=12/dose group) were administered vehicle control, 30, 60 or 100 mg/kg ibogaine i.p. as a single dose. Two animals in the 100 mg/kg group died within one day. Observations included clinical pathology, and histopathology and neuropathology of the sciatic nerve. None of the hematological or clinical biochemical values showed statistically significant changes from vehicle control. In addition, in the nerve conduction studies, neither the average conduction velocity nor the area under the curve, showed an ibogaine-treatment effect. There were no neuropathologic lesions seen under light microscopy that were considered to be related to ibogaine. In this study, doses of ibogaine that were acutely toxic did not produce pathology or peripheral neurotoxicity. **Supported by NIDA Contract No. NO1DA-2-9307.**

679.4

EFFECTS OF FUMONISIN B1 ON BLOOD-BRAIN SPHINGANINE TURNOVER AND FB1 TOXICOKINETICS IN THE DEVELOPING RAT. O. S. Kwon and W. Slikker, Jr*. Dept. of Pharm./Toxicol., Univ. of Arkansas for Medical Sciences, Little Rock, AR and Div. of Neurotoxicol., National Center for Toxicological Research, FDA, Jefferson, AR.

Fumonisins are toxic metabolites that are produced by *Fusarium moniliforme* in corn. Fumonisin B1 (FB1) has been reported to increase sphinganine (Sa) in kidney, liver and serum from rodents and other mammals. Our previous results showed that FB1 increases Sa levels in the brain and spinal cord and reduces myelin deposition in rat forebrain. Increase of Sa in blood may produce a pool of Sa available for transport to the brain. The objective of this experiment was to elucidate whether blood Sa has effects on the brain Sa turnover. FB1 and Sa levels both in brain and plasma were measured by HPLC from postnatal day(PND) 12 rats treated with a single dose of FB1 (sc, 0.8 mg/kg or 8 mg/kg). Area under the curve (AUC, 0 to 24 hr) of Sa levels was remarkably higher in forebrain (124.1 nmol/g.hr) than in plasma (3.1 nmol/ml.hr) after 8 mg/kg FB1. AUC ratios of brain to plasma Sa concentration curves following acute administration were 37.2 at 0.8 mg/kg FB1 and 39.7 at 8 mg/kg FB1. The AUC ratio of brain to plasma FB1 was 0.03. Observations from plasma FB1 disappearance curves over a 12 hr period indicated that the plasma half life of FB1 in PND 12 rats is 1.2 to 1.7 hr, and the volume of distribution is about 0.2 l/kg. These data indicate that even low bioavailability of FB1 in the brain is sufficient to produce the striking increase of Sa levels in the brain. These data also suggest that elevations of brain Sa levels are related to direct action of FB1 on the brain rather than the transfer of blood Sa into the brain.

679.5

INTERLEUKIN-1 AND TUMOR NECROSIS FACTOR-α SYNERGISTICALLY MEDIATE NEUROTOXICITY VIA NO AND NMDA RECEPTORS. S. Hu*, L. Erlich, P. K. Peterson and C. C. Chao. Minneapolis Medical Research Foundation and the University of Minnesota Medical School, Minneapolis, MN 55404

The pro-inflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF)-α, produced by glial cells within the brain, play a pathogenetic role in several neurodegenerative diseases; however, little is known about the mechanisms underlying cytokine-mediated neurotoxicity. Using human fetal neuronal cell cultures, we investigated the injurious effect of these cytokines. Although neither cytokine alone was toxic, IL-1β (1 ng/ml) and TNF-α (20 ng/ml) in combination for 7 days caused marked ($P<0.01$) neuronal injury, as assessed by increasd release (180% over control) of lactate dehydrogenase (LDH), a marker of neuronal loss. In the presence of TNF-α (20 ng/ml), 1 ng/ml of IL-1β caused maximal neuronal death. The ED_{50} of IL-1β was approximately 300 pg/ml in TNF-α-treated neuronal cell cultures. The neurotoxic effect of these two cytokines was synergistic, and antibodies (10 μg/ml) to IL-1β, TNF-α or IL-1α blocked 94%, 97% and 9% of LDH release, respectively. Blockade of nitric oxide (NO) production with the NO synthase inhibitor was accompanied by marked ($P<0.01$) reduction (about 45%) of cytokine-mediated neuronal injury, suggesting that NO is partly involved in mediating cytokine-induced neurotoxicity. Addition of the N-methyl-D-aspartate (NMDA) receptor antagonists to neuronal cell cultures blocked ($P<0.01$) cytokine-mediated neurotoxicity by >55%, suggesting the involvement of NMDA receptors. Also, treatment of neuronal cell cultures with IL-1β plus TNF-α inhibited ($P<0.01$) the high-affinity ^{3}H-glutamate uptake and astrocyte glutamine synthetase activity, two major pathways involved in protection against NMDA receptor-mediated neurotoxicity. These *in vitro* findings may lead to development of new therapeutic strategies for neuronal damage in cytokine-mediated neurodegenerative diseases.

679.6

MODULATION OF SEROTONIN-INDUCED CURRENTS BY METALS IN MOUSE NEUROBLASTOMA CELLS. M. Uki* and T. Narahashi. Dept. of Mol. Pharmacol. and Biol. Chem., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The effects of several metals on the serotonin receptor-channel complex were studied using mouse neuroblastoma N1E-115 cells which are known to be endowed with the 5-HT_3 subclass of the receptor. The whole-cell patch clamp technique was used to record currents induced by serotonin at a concentration of 3 μM which was equivalent to the apparent dissociation constant. Methylmercury and mercuric chloride suppressed serotonin-induced currents irreversibly with IC_{50}s of 3 μM and 2 μM, respectively. Lead and zinc suppressed the current with IC_{50}s of 80 μM and 50 μM, respectively, and the effects of both metals were reversible after washing with metal-free solution. Lanthanum also suppressed the current with an IC_{50} of 10 μM, and the effect was partially reversible. Cadmium and cobalt augmented serotonin-induced currents slightly but consistently at a concentration of 100 μM, and the effect was reversible. Aluminum at 100 μM had no effect on serotonin-induced currents. It was concluded that the 5-HT_3 receptor is endowed with a unique property with respect to the actions of metals which is not shared by some other ligand-gated and voltage-gated ion channels.

679.7

BILIRUBIN INDUCES APOPTOSIS OF CEREBELLAR NEURONS VIA ACTIVATION OF NF-KB. G.-M. Yan[1,2]*, S.-Z. Lin[1], J.Gu, R.P. Irwin[1] & S.M. Paul[1,2] [1]Dept. of Pharmacol. & Toxicol., Sch. of Med., IUPUI, Indianapolis, IN 46202; [2]Lilly Res. Labs, Eli Lilly & Company, Indianapolis, IN 46285

Bilirubin, a degradation product of protoporphyrins derived from hemoglobin, is a well known neurotoxin. However, neither the cellular nor molecular mechanisms underlying bilirubin-induced neurotoxicity are known. We now report that low concentrations of bilirubin are toxic to cultured rat cerebellar neurons. By contrast, cultured fetal rat cortical or hippocampal neurons are relatively insensitive to bilirubin toxicity. Moreover, bilirubin-induced toxicity of cerebellar neurons is associated with the biochemical and morphological features of apoptosis. Bilirubin-induced apoptosis is blocked by RNA and protein synthesis inhibitors, suggesting a gene-directed mechanism. Using gel shift (EMSA) analysis we found that bilirubin activates the transcription factor NF-κB in a concentration- and time-dependent manner in both intact cultured cerebellar granule neurons and cell-free extracts. Moreover, TPCK and TLCK, specific inhibitors of NF-κB, block both bilirubin-induced apoptosis of cerebellar granule neurons and bilirubin-induced NF-κB activation. These data strongly suggest that activation of NF-κB is the initial intracellular event mediating bilirubin-induced apoptosis of cerebellar neurons. NF-κB may play a more general role in neuronal apoptosis as well.

679.8

NEUROHISTOLOGICAL AND NEUROBEHAVIORAL ASSESSMENT OF THE ANTI-HIV THERAPEUTIC 2',3'-DIDEOXYINOSINE (DDI) IN RATS. T.A. Patterson*, J.A. Sandberg, L.C. Schmued, C.M. Albertson, M.G. Paule and W. Slikker, Jr. Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079-9502.

The anti-HIV therapeutic dideoxyinosine (ddI) has been reported to produce a painful, dose-limiting peripheral neuropathy in HIV-infected patients after chronic administration. However, distinguishing this toxicity from the disease itself is complicated. Therefore, the nature of this peripheral neuropathy was examined in a non-HIV infected animal model. Rats (N=9/group) were gavaged with vehicle (VEH), 41.5 or 415 mg/kg ddI, or 41.5 mg/kg isoniazid (INH; as a positive control) twice daily for 20 weeks. Plasma ddI levels peaked by 30 min and were constant over the first 1.5 hr after administration, averaging (±SD) 1.05 ± 0.41 and 3.97 ± 2.57 μg/ml for the low and high dose, respectively. Average INH plasma concentrations during the same time period were 9.85 ± 7.21 μg/ml. Schedule-controlled operant behaviors, motor assessments, nerve conduction velocities and pathologies were examined. Mortality was dose and drug dependent: high ddI (44%) > INH (33%) > low ddI (0%) = VEH (0%). Ataxia and seizure activity were first noted in the INH group after 11 weeks of dosing. In rats treated with INH, myelin splitting, whorls, extracellular debris, mast cells, and reduced axonal number were observed in sciatic nerve sections. These findings were consistent with the behavioral manifestations of peripheral neuropathy. After chronic ddI administration, there were no nerve conduction or behavioral alterations, but histological analysis revealed myelin splitting and intramyelin edema. Thus, rats chronically dosed with ddI exhibited peripheral nerve myelinopathy, even in the absence of HIV infection. **(supported by NIEHS IAG #Y01-ES-10187)**

679.9

LACK OF PROTECTIVE EFFECTS OF SIGMA LIGANDS ON THE INDUCTION OF HEAT SHOCK PROTEIN HSP-70 IN RAT CEREBROCORTICAL NEURONS BY DIZOCILPINE. K.Hashimoto*, S.Tomitaka, N.Narita, Y.Minabe, M.Iyo and S.Fukui. Natl. Inst. Neurosci., NCNP, Tokyo 187 and NIMH, NCNP, Chiba 272, Japan.

The non-competitive NMDA receptor antagonists such as MK-801 (dizocilpine) and phencyclidine injure a discrete populations neurons in the posterior cingulate and retrosplenial cortex of rat brain. These drugs produce vacuolization and necrosis in these neurons. Sharp et al. (1992) have reported that these drugs induce production of heat shock protein HSP-70, and that antipsychotic drug haloperidol and sigma agent rimcazole protect the induction of HSP-70 protein by dizocilpine and phencyclidine, suggesting a role of sigma receptors in the expression of HSP-70 protein by NMDA antagonists. We studied the role of sigma receptors on the induction of HSP-70 protein by dizocilpine. Dizocilpine (1 mg/kg, i.p.) was injected into female SD rats. The immunocytochemistry of HSP-70 was studied 24 hr after administration of dizocilpine. Sigma ligands such as 4-PPBP (3 mg/kg) and NE-100 (3 mg/kg) were administered 15 min before injection of dizocilpine. The treatment with dizocilpine produced the induction of heat shock protein HSP-70 in the posterior cingulate and retrosplenial cortex of rat brain. However, the pretreatment with 4-PPBP and NE-100 could not attenuate the induction of HSP-70 protein by dizocilpine. Thus, it is unlikely that sigma receptors may play a role in the induction of heat shock protein HSP-70 by NMDA receptor antagonists.

679.10

NEURONAL CULTURE MODEL OF CENTRAL NERVOUS SYSTEM OXYGEN TOXICITY. H. T. Whelan *, D. M. Bajic, and E. P. Kindwall. Neurology and Hyperbaric Medicine, Medical College of Wisconsin, Milwaukee, WI 53226, & NAVMARCORESCEN, Milwaukee, WI 53207.

Hypothesis: Plasma membranes are important in the transport processes of neurotransmitters regulating neuronal excitability during seizures caused by hyperbaric oxygen (HBO). The CNS becomes irreparably damaged if HBO is continued. These neurochemical effects may be preventable using the 21-aminosteroid U74389G, which reportedly has an effect of inhibiting neuronal membrane lipid peroxidation. Diazepam binding reflects neuronal cell membrane integrity. Peroxidation of the cell surface membrane should alter diazepam binding. **Method:** To assess neuronal membrane changes due to HBO, neuron cultures were subjected to 100% oxygen at 1.5 ATA. Control cultures were compared to those pretreated with 21-aminosteroid. Diazepam binding was used as a marker of surface membrane integrity. Fetal mouse neurons at 3.5×10^6 cells/ml were treated with clonozepam and ^{3}H-diazepam. The clonazepam-displaceable (neuron-specific) diazepam binding was assessed by scintillation counting, according to published methods. **Results:** Neurons subjected to HBO or to 21-aminosteroid alone developed 30% inhibition of diazepam binding, reflecting surface membrane dysfunction in this model. Neuronal surface membrane integrity (clonazepam-displaceable diazepam binding) was completely protected from HBO by pretreatment with 21-aminosteroid. The reason for lack of diazepam binding when 21-aminosteroid is given alone is not understood. This neuron culture model may be useful for testing pharmacologic means of protecting surface membranes in conditions of hyperbaric oxygen toxicity.

679.11

DOMOIC ACID: ASTROCYTIC, NEURODEGENERATIVE AND BEHAVIORAL RESPONSES. A.C. Scallet*, L.C. Schmued, R.L. Rountree, J.N. Johannessen[1], and T.J. Sobotka[1]. NCTR/FDA, Jefferson, AR and [1]CFSAN/FDA, Laurel, MD.

Four groups of 6 adult male rats were treated i.p. with domoic acid (0, 0.22, 0.66, or 1.33 mg/kg). Eight days later, after behavioral testing, rats were perfused. Hippocampal sections were stained for neurodegeneration and for glial fibrillary acidic protein (GFA). Only a subset (2/6) of the high-dose group had extensive damage, mainly in CA1. GFA-positive astrocytes in CA1 were larger in high dose rats than controls (107 ± 26 vs. 53 ± 7 sq microns, $p < 0.05$, mean ± SEM of 6 cells per rat). The 2 rats with CA1 damage also had the most enlarged astrocytes. These same two rats were the most hypophagic after dosing, were poor at passive avoidance performance, and exhibited exaggerated startle responses, suggesting the presence of a behavioral hyperreactivity syndrome. There was a smaller, but still dose-related increase in astrocyte size even at domoic acid levels not causing neurodegeneration, "reactive" gliosis, or behavioral effects. Our data indicate correlations between neurohistological and neurobehavioral biomarkers of domoic acid exposure, and emphasize the potential importance of astrocytic response to low-dose exposure.

679.12

APOPTOTIC CELL DEATH INDUCED BY THE NEUROTOXIN ETHYLCHOLINE AZIRIDNIUM (AF64A) IN VITRO AND IN VIVO. H.Hörtnagl*, W.Rinner, C.Pifl and H.Lassmann. Institute of Biochem. Pharmacology and Institute of Neurology, Univ. Vienna, A-1090 Vienna, Austria.

Increasing evidence indicates the significance of programmed cell death for neurodegenerative diseases. Various neurotoxins induce neuronal cell death by induction of apoptosis, at least in vitro. In this study we focussed on the mechanisms of cell death induced by the neurotoxin ethylcholine aziridinium (AF64A) in vitro and in vivo. The in vitro effect was compared with that of the aziridinium derivative of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4). For the in vivo study male Sprague Dawley rats (400-500g) received stereotaxical infusion of 1 or 2 nmol AF64A into the left and the corresponding vehicle into the right lateral ventricle. Rats were perfused under deep chloral hydrate anesthesia with 4% paraform-aldehyde 1, 2, 3 and 7 days after AF64A application. Apoptotic features including nuclear chromatin condensation and DNA fragmentation as revealed by in situ nick translation was observed in various parts of the hippocampus, adjacent parietal cortex and septum, with a maximum after 2 to 3 days. Both neuronal and glial cells were affected. For in vitro evaluation human neuroblastoma cells SK-N-MC and human embryonic kidney cells 293 were used. A dose- and time-dependent increase in the number of apoptotic cells resulted from the exposure to AF64A (50 and 100nM) or DSP4 (10 - 100 nM) in the medium. Apoptotic changes started after 8 h of exposure and reached a maximum between 15 and 24 h. In addition to light microscopic observations the characteristic features of apoptotic cell death were identified by electron microscopy. Apoptosis was suppressed by inhibition of endonuclease by Zn (800µM) and by the free radical scavenger Tempol (2mM). The data indicate that apoptosis contributes to the neurodegeneration induced by neurotoxins containing an aziridinium moiety and support their use in models for neurodegenerative diseases.

679.13

THE LOCALIZATION OF 3-NITROPROPIONIC ACID (3-NPA) INDUCED NEURONAL DEGENERATION IN ADULT RATS. Z. Binienda*, R.L. Rountree, L.C. Schmued and A.C. Scallet. NCTR/FDA, Jefferson, AR 72079-9502.

The localization of neuronal degeneration following chronic treatment with the mitochondrial energy metabolism inhibitor 3-NPA was determined in adult male Sprague-Dawley rats using a silver degeneration method (Nadler-Evenson) and a novel fluorescent technique. Rats were injected intraperitoneally with escalating doses of 3-NPA (5 mg/kg/day to a maximum 30 mg/kg/day; Monday-Friday). Rats were perfused under deep pentobarbital sodium anesthesia with 4% formaldehyde in phosphate buffer (0.1 M, pH 7.4). Coronal sections were stained using silver impregnation technique specific for degenerating axons, terminals and neurons. Although some variability in the distribution of lesions was observed, areas most frequently damaged included neurons of the cingulate and insular cortex, patches of CA1 hippocampal pyramidal neurons, a considerable extent of the axons within the ventral thalamus, a variable portion of the caudate nucleus and neurons of the deep nuclei of the cerebellum. We hypothesize that the pattern of neurodegeneration produced by 3-NPA in the forebrain and the cerebellum relates to a selective sensitivity to energy depletion of these regions.

679.14

COMPARISON OF VESTIBULAR DYSFUNCTION AND PERIPHERAL NEUROPATHY FOLLOWING SUBCHRONIC DRINKING WATER EXPOSURE TO 3,3'-IMINODIPROPIONITRILE (IDPN) IN THE RAT. J.Llorens, K.M.Crofton*[1] and E.Rodríguez-Farré. Dept. Pharmacol. Toxicol., CSIC, 08034 Barcelona, Spain and [1]Neurotoxicology Division, US EPA, Research Triangle Park, NC 27711, USA.

IDPN induces neurofilament (NF)-filled swellings in the proximal region of large caliber axons, and degeneration of vestibular sensory hair cells which causes a syndrome of abnormal behavior. Following acute exposure, vestibular toxicity appears at doses lower than those required to induce overt neuropathic effects. The present study compares vestibular and peripheral nerve effects of IDPN following subchronic exposure. Adult Long-Evans rats were exposed to 0, 0.025, 0.05, 0.1 or 0.2 % IDPN in the drinking water for 13-15 weeks. Exposure was discontinued after 7 weeks in a group receiving 0.4 % IDPN due to marked body weight loss. Motor activity was assessed at 0, 1, 3, 6, 9 and 12 weeks of exposure, and vestibular function at 0, 1, 2, 4, 6, 9, and 12 weeks. The vestibular system and the dorsal root ganglia (DRG) were assessed for pathological changes at the end of the exposure. Effects on body weight, motor activity, vestibular function and vestibular morphology were observed after 0.2 and 0.4 % IDPN. Axonopathic effects in the DRG were obvious after 0.2 %. Thus, during subchronic exposure to IDPN, proximal axonopathies do not require larger dosages than vestibular toxicity, in contrast to acute exposure.

679.15

NEUROTOXICITY OF SERUM FROM PATIENTS WITH INSULIN-DEPENDENT DIABETES (IDDM) IS ASSOCIATED WITH INCREASED TRK-A EXPRESSION AND IS PARTIALLY REVERSED BY NGF. Gary L. Pittenger, Dong Liu, Pauline G. Newlon*, Aaron I. Vinik. Depts. of Internal Medicine, Surgery and Anatomy & Neurobiology, Eastern Virginia Medical School, Norfolk, VA 23510

We have previously demonstrated that immunoglobulins (Ig) from serum of IDDM patients with neuropathy are toxic to the clonal adrenergic N1E-115 neuroblastoma cell (NB), suggesting that autoimmunity contributes to diabetic neuropathy. In experimental autoimmune neuropathy, nerve growth factor (NGF) has been shown to be protective. In these studies, we tested whether NGF might protect NB from the neurotoxic effects of IDDM serum. After plating equally into 35 mm^2 dishes, NB were exposed to 10% of either control subject (n=6) or IDDM patient serum (n=6) and, separately, to the sera plus increasing concentrations of NGF from 100 ng/ml to 2 μg/ml. After 72 h the cultures were lifted from the dishes and viable cells counted by trypan blue exclusion criteria. In the presence of NGF and IDDM serum, cell numbers increased in a dose-dependent manner, even in the face of the most toxic IDDM serum which killed all cells. On average, the optimal dose was 500 ng/ml. The effects of the IDDM sera were only partially reversed by NGF. In order to test whether IDDM serum causes alterations in trk A gene expression, *in situ* hybridization was performed on NB using a probe (Genentech, San Francisco) specific to trk A. The results indicated that NB make trk A RNA and that the expression of trk A receptor gene is increased 2-3 fold in response to IDDM serum, but not in response to control subject serum. In summary, NGF has a dose-dependent, partially protective effect on N1E-115 cells treated with neurotoxic IDDM serum. The neurotoxic effect is not due to down-regulation of trk A receptor gene. Further studies are required to determine any role of altered NGF/receptor signalling in the neurotoxicity of IDDM serum.

679.16

CYTOKINES AND DELIRIUM: AN EXPLORATORY STUDY. J.R. Mach, D.T. Weldon, N. L. Opstad, K.M. Bettin, and J.A. Mortimer*. Geriatric Research Education Clinical Center (GRECC) (11G), VA Medical Center, Minneapolis, MN 55417

Delirium occurs in 20-30% of older inpatients and is associated with a mortality rate nearly twice that of controls. Despite its clinical significance, the pathophysiology of delirium is virtually unknown. Delirium consists of behavioral and cognitive symptoms caused by a variety of conditions that are not primary CNS diseases. Cytokines have been found to mediate many disease-brain interactions, such as eating disorders, stress reactions, cancer, and infections. To date, the role of cytokines in delirium has not been studied. This study examined the relationship between delirium and serum levels of selected "pro-inflammatory" cytokines IL-1α, IL-1β, IL-2, IL-6, and TNF-α.

Twelve delirious patients were recruited from the inpatient wards at the Minneapolis VA Medical Center. Subjects met diagnostic criteria for delirium using the CAM and were age 55 or older. Patients with pre-existing dementia were excluded. Eight nondelirious inpatient controls, each matched for age and illness severity to a delirious subject, were enrolled for comparison. Serum cytokine assays were performed using ELISA kits from R & D Systems. Wilcoxon matched-pairs signed-ranks test for non-parametric distributions was used to analyze the two sets of serum cytokine data. IL-6 serum levels (pg/ml) declined significantly in the delirious group as clinical symptoms improved (mean ± SE = 40.75 ± 12.24 vs. 20.25 ± 7.99, respectively, $p < 0.05$). Furthermore, IL-6 was significantly elevated in the delirious group compared to matched controls (49.47 ± 12.36 vs. 16.25 ± 5.16, respectively, $p < 0.02$). Serum levels of IL-1α, IL-1β, IL-2, and TNF-α were below detection limits in delirious subjects and controls. These results suggest that IL-6 may play a role in delirium pathophysiology. Supported by a grant from the Department of Veteran Affairs.

679.17

NEUROVIRULENT SIMIAN IMMUNODEFICIENCY VIRUS STRAIN INDUCES APOPTOSIS IN THE CENTRAL NERVOUS SYSTEM. D.C. Adamson*, T.M. Dawson, M.C. Zink, J.E. Clements, & V.L. Dawson. Neurology, Neuroscience, & Comp. Med., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21202.

Studies of human immunodeficiency virus type 1 (HIV-1) infection in the central nervous system (CNS) have shown neuronal loss in discrete areas; however, the presence and mechanism of neuronal death, particularly in the case of HIV-1 associated dementia has remained quite elusive. Programmed cell death or apoptosis, has been clearly demonstrated outside the CNS in HIV-1, but has not been firmly established within the CNS. Using a simian immunodeficiency virus (SIV) animal model for HIV-1 CNS infection, we investigated whether SIV infection of the CNS is spatially associated with apoptosis of neurons and non-neuronal cell types. With the aid of an *in situ* technique for identifying the 3'-OH ends of newly fragmented DNA characteristic of apoptosis, we demonstrate the presence of apoptotic neurons, astrocytes, endothelial cells, oligodendrocytes and microglia. We also observe the colocalization of apoptotic cells with numerous perivascular inflammatory cell infiltrates and SIV-infected cells. Quantitative analysis reveals significantly more apoptotic CNS cells in neurovirulent SIV strains as compared to strictly lymphocyte-tropic strains and SIV negative controls. Our findings of SIV induced apoptosis in the CNS will hopefully lead to a better understanding of sequelae of HIV-1 CNS infection such as HIV-1 associated dementia.

679.18

SELECTIVE KILLING OF CHOLINERGIC NEURONS BY MICROGLIAL ACTIVATION IN CELL CULTURE

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LEN/ NIEHS/ NIH, RTP, NC 27709

Inflammation may be important in the etiology of Alzheimer's disease and cholinergic neurons are lost early in the course of this disease. We previously showed that dopaminergic neurons in mixed mesencephalic cultures are selectively killed by activation of microglia by lipopoly-saccharide (LPS) and that production of nitric oxide (NO) is critical for neurotoxicity. LPS (10 μg/ml) treatment (2 days) also killed most choline acetyltransferase-positive (Chat+) neurons (50-80%) in fetal rat basal forebrain cultured on hippocampal glia; unstained neurons were spared. Inducible NO synthase was visible in microglia and the levels of nitrite (the NO metabolite) were increased 2 days after LPS addition. The NO synthase inhibitor NAME (0.5 mM) protected the Chat+ neurons. The opiate morphine and the anti-inflammatory steroid dexamethasone inhibited LPS-induced NO formation in these cultures, and may have cholinergic neuroprotective effects. β-amyloid, the HIV protein GP120 and γ-interferon all increased NO, and microglial activation may explain their cholinergic neurotoxicities.

679.19

EVALUATION OF THE EFFECTS OF FLUOXETINE ON MOTOR ACTIVITY IN RATS. Bruce Culver* and Shannon Howrey School of Pharmacy, University of Wyoming, Laramie, WY 82071.

Akathesia and other motor dysfunctions are side effects associated with the use of fluoxetine (FL). The mechanisms underlying these motor effects remain poorly understood. Acute administration of FL in rats has been suggested as an animal model of akathesia (Teicher and Wallace, Neurosci Abst, p. 1868, 1993). Our studies were designed to further characterize motor effects of rats injected with FL (30mg/kg; s.c.) using 2 different methods of activity testing. Measures of photocell counts recorded over 2-4 days from groups of female rats in a figure 8 maze revealed rats injected with FL had less locomotor activity during the exploratory periods than paired saline control groups. Decreases in activity were also recorded from FL-treated rats during the rest of the diurnal and nocturnal periods. Digiscan (Omnitech) activity monitors were used to provide several different measures of motor activity of individual rats placed in the units for a 6 hr period. Female rats treated with FL generally showed a decreased total distance traveled consisting of non-stereotyped decreases in horizontal and vertical activities compared to controls, but differences were significant only during the first 2 hrs in the apparatus. However, males treated with FL showed an increase in total distance moved after 3 hrs in the units and the activity consisted of increased stereotypy and horizontal movements. Our findings that FL generally decreases most motor activities of female rats seems to be inconsistent with previous reports of FL-induced increased locomotor activity and "restlessness", which may correlate with our findings of certain increases in motor activity in FL males. Additional characterization of motor behaviors is needed to evaluate the utility of this model of FL-induced motor dysfunction.

679.20

UPREGULATION OF HIGH AFFINITY NEUROTROPHIN RECEPTOR, TRK B-LIKE PROTEIN ON WESTERN BLOTS OF RAT CORTEX AFTER CHRONIC ETHANOL TREATMENT(CET). I. Baek,* M.B. Heaton & D.W. Walker, Dept. of Neuroscience, College of Medicine, Univ. of Florida, Gainesville, FL 32610

We previously reported that the total neurotrophic activity of hippocampal extracts was significantly (25-50%) reduced after 21-28 wks of CET suggesting that the level of one or more neurotrophic factors may be compromised in CET rats (Walker et al. 1992, Neurosci. Lett. 147:77-80). Such compromised level of neurotrophic factor(s) (i.e., ligand itself), changes in receptors for the neurotrophic factors, or combination of both may induce the neuronal degeneration observed in neurotrophin-dependent neurons after CET.

To investigate possible changes in neurotrophic factor-receptors, we examined Western blots of rat cortex after 28 wks of CET. After sonication and ultra-centrifugation, the supernatant of crude lysates of individual animals' cortex was subjected to SDS-PAGE, electrotransfer to nitrocellulose membrane, incubation with α-*trk* B Ab and alkaline phosphatase-conjugated protein A, and chemiluminescent substrate reaction. The membranes were then exposed to Kodak XAR film.

Compared to controls (n=6), CET rats (n=7) appeared to have significantly higher intensity of *trk* B-like protein at about 145 KD, which suggests up-regulation of *trk* B to compensate the compromised level of certain subset (i.e., BDNF, NT-3, 4 or 5, but not NGF) of neurotrophins in cortex.

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679.21

DECREASED CROSS-SECTIONAL AREA OF THE CORPUS CALLOSUM IN YOUNG FEMALE ALCOHOLICS: AN MRI STUDY D Hommer*, R. Momenan, P. Ragan, W. Williams, D. Rio, M. Eckardt National Institute on Alcohol Abuse and Alcoholism, DICBR, Laboratory of Clinical Studies, Bethesda, MD 20892-1256

Although, females appear to be more sensitive to the hepatic consequences of alcoholism, to date, there has been no evidence that females suffer more severe structural CNS sequelae of alcoholism. Several groups have used magnetic resonance imaging (MRI) techniques to compare brain structure between alcoholics and controls. These studies have primarily examined males. We compared cross-sectional area of the corpus callosum in a group of 14, hospitalized, alcoholic women (mean age = 38.9±6.7 years) with a group of 9 non-alcoholic women (35.9±4.7 years). The corpus callosum and the inner table of the skull were outlined by hand on a mid-saggital T_1 weighted MRI image. There was no difference in intra-cranial area between the alcoholics and controls; however, corpus callosum area was significantly smaller among the alcoholics (717±78 mm^2 versus 566±105 mm^2, $t(21)=3.70$, $p < .002$). When the corpus callosum was divided into four segments of equal length, the reduction in area did not appear to be localized. Alcoholic males did not differ from control males in corpus callosum or intra-cranial area. These results may indicate an increased sensitivity to alcohol-induced brain damage among females.

679.22

METHANOL TOXICITY: SPECIES DIFFERENCES IN RETINAL FORMATE OXIDATION. J. T. Eells *, A. M. Flaig, J. J .Schultz, and M. M. Salzman. Department of Pharmacology and Toxicology. Medical College of Wisconsin, Milwaukee, WI 53226.

Formic acid is the toxic metabolite responsible for the retinal and optic nerve toxicity produced in methanol poisoning. The primary site of formate oxidation and detoxification to CO_2 is the liver. However, there is little information regarding formate oxidation in the retina, an important target tissue of formate toxicity. The purpose of this study was to compare the capacity of human retinal tissue and retinal tissue from a methanol-resistant species, the rat, to oxidize formate. Formate oxidation was measured *in vitro* in isolated human and rat retina. The oxidation of ^{14}C-formate to $^{14}CO_2$ by human and rat retinal tissue followed pseudo-Michaelis-Menten kinetics. The pseudo-K_m did not vary between the two species and averaged 2.3 mM. However, the maximum rate of formate oxidation (pseudo-V_{max}) was 2-times higher in rat retinal tissue (63 ± 8 nmole CO_2/g/min) than in human retinal tissue (29 ± 3 nmole CO_2/g/min). The apparent *in vitro* metabolic half-life of formate was calculated to be 31 min. in rat retina and 54 min. in human retina. Humans and non-human primates are uniquely sensitive to methanol toxicity. These data support the hypothesis that species differences in retinal formate oxidation may play a role in the susceptibility to methanol poisoning. (Supported the American Petroleum Institute and NIH grant ES06648).

679.23

CATALASE OXIDATION OF ETHANOL TO ACETALDEHYDE IN FETAL BRAIN MAY CONTRIBUTE TO THE TOXIC EFFECT OF MATERNAL ALCOHOL CONSUMPTION. R. Hamby-Mason[1], B. Cobb-Alls[2]* and G. Henderson[1]. [1]University of Texas Health Science Center, San Antonio, TX 78284 and [2]Armstrong Laboratory, Radiofrequency Radiation Division, Brooks AFB, TX, 78235.

Previous studies documenting oxidative stress in rat fetuses exposed to ethanol (E) *in utero* have shown an increase in catalase activity (Cat). This E-enhanced Cat, combined with elevated Cat in fetal/neonatal brain, may increase acetaldehyde (AcHO) formation in excess of that in the adult. Both Cat and AcHO production from E were assayed in 10% homogenates of fetal, neonatal and adult brain. Aliquots of the homogenates were incubated (60 min) with 20 mM E. AcHO levels (by HPLC) and Cat (spectrophotometric) were determined. Addition of E to adult brain homogenates increased AcHO by 251% above the control (C) and elevated Cat by 34.1%. Pretreatment with Cat inhibitors, sodium azide and 3-amino-1,2,4 triazole significantly decreased production of AcHO by E. Neither 4-methylpyrazole, an alcohol dehydrogenase inhibitor, nor isoniazid, a P450 inhibitor, had an effect on the amount of recovered AcHO. Cat was highest in brains from 17 and 19 day old fetuses (17.01±1.27 and 11.40±2.40 SE, units) and declined to 8.15±0.42 units for day one neonates and to 3.6±1.4 units for adults. Neonatal brains more rapidly metabolized E to AcHO (379% vs 251% above C) than did adult brain. Exposure to E *in utero* increased brain Cat in day 17 fetuses at 1 and 3 hrs post E by 55.3% and 92.9% respectively, with a 447% increases in AcHO production at 3 hrs. This indicates that high basal levels of Cat in immature brain, combined with E-enhanced Cat, increase production of AcHO at a rate above that in adult brain. Thus, enhanced Cat oxidation of E may be an important mechanism underlying the toxic effects of maternal E consumption on the fetal brain.

680

SYMPOSIUM. MOLECULAR ORGANIZATION OF THE POSTSYNAPTIC MEMBRANE. Mary B. Kennedy, Caltech (Co-chair); Justin R. Fallon, Worcester Foundation; Heinrich Betz, Max-Planck Institute, Frankfurt; Stanley C. Froehner (Co-chair), Univ. of North Carolina at Chapel Hill.

The mechanisms leading to the formation of postsynaptic specializations are most completely understood at the neuromuscular junction. Although the heterogeneity of central nervous system synapses makes them less amenable to biochemical studies, recent advances have revealed features in common with the neuromuscular junction (e. g., the presence of members of the dystrophin protein family and associated proteins) as well as important differences. This symposium will emphasize the emerging principles common to neuromuscular and central synapse formation, as well as those molecules and mechanisms specific for each synaptic type. **Fallon** will discuss the characterization of the agrin receptor and the mechanism by which agrin initiates AChR clustering. **Froehner** will describe proteins of the neuromuscular postsynaptic cytoskeleton, including the 43K protein and components of the dystrophin/utrophin complex, that appear to anchor ACh receptors at synaptic sites.Some proteins associated with glutamatergic and glycinergic synapses in the CNS are not found at the neuromuscular junction and probably mediate specialized functions. **Betz** will present data on the protein gephyrin, which is essential for clustering of glycine receptors in spinal cord neurons, probably via a mechanism involving microtubules. Finally, **Kennedy** will discuss the postsynaptic density at glutamatergic synapses, which contains signal transduction molecules that appear to play a specialized role in mediating forms of synaptic plasticity that are unique to these synapses.

681

SYMPOSIUM: DEVELOPMENTAL DETERMINANTS OF RETINAL GANGLION CELLS. L. M. Chalupa, UC Davis (Chairperson); R. O. L. Wong, Washington University; A. E. Hendrickson, University of Washington; R. W. Guillery, Oxford University.

This symposium will deal with diverse mechanisms regulating the formation of some of the key features of retinal ganglion cells and their precise patterns of projections. Wong will discuss correlated retinal activity patterns demonstrated by multi-electrode arrays and optical recording techniques in relation to spontaneous activity and neurotransmitter responsivity of developing ganglion cells. Chalupa will discuss the roles of retinal afferents and sodium voltage-gated activity in regulating the establishment of On and Off ganglion cell mosaic patterns in the postnatal cat retina. Hendrickson will present results of light and EM studies, as well as immunocytochemical experiments, directed at assessing the factors responsible for the density shifts that give rise to the primate fovea. She will consider such issues as the suppression of rods and blue cones in the foveal region, and the effects of cell migration on the state of synaptic contacts formed prior to the differentiation of the fovea. Guillery will talk about stages of the guidance mechanisms that lead axons into a crossed or an uncrossed pathway at the optic chiasm, and will relate this to ganglion cell classes identified in cats and ferrets.

684.1

SUB-THRESHOLD SYNAPTIC ACTIVATION OF Ca^{2+} CHANNELS MEDIATES A LOCALIZED Ca^{2+} INFLUX INTO DENDRITES OF HIPPOCAMPAL PYRAMIDAL NEURONS. J. C. Magee*[1,2], G. Christofi[2], H. Miyakawa[2], B. Christie[1,2], N. Lasser-Ross[2], & D. Johnston[1,2], [1]Div. Neurosci., Baylor Coll. of Med., Houston, TX and [2]MBL, Woods Hole, MA

Hippocampal CA1 pyramidal neurons contain a relatively high density of TTX-sensitive Na^{2+} channels and at least three types of Ca^{2+} channels. Of these Ca^{2+} channels, the LVA or T-type channel in the dendrites can be activated by sub-threshold EPSPs. Such channel activation could greatly impact the dendritic integration of synaptic activity. Furthermore, if sub-threshold voltage-gated channel activation provides a significant influx of Ca^{2+}, such channels could participate in the long-term regulation of synaptic efficacy. We used whole-cell patch-clamp techniques in conjunction with high-speed fluorescence imaging of fura-2 to characterize subthreshold, synaptically activated Ca^{2+} influx into the dendrites of CA1 pyramidal neurons. We consistently observed a significant non-NMDA (50 μM APV included in bath) mediated elevation of dendritic intracellular Ca^{2+} concentration in response to sub-threshold synaptic stimulation (5 stimuli at 50 Hz). These Ca^{2+} signals were localized to branches of the dendritic arborization that received active synaptic input. Prior membrane hyperpolarization (15–25 mV for 1.5–2 secs, 250–400 ms before synaptic stimulation) enhanced the Ca^{2+} influx by up to 66 ± 10% ($n = 5$). 50 μM $NiCl_2$ reduced the amplitude of this postsynaptic influx of Ca^{2+} by 54 ± 5% ($n = 8$). These data suggest that Ni^{2+}-sensitive, voltage-gated Ca^{2+} channels with relatively hyperpolarized voltage-ranges of activation and inactivation mediate a substantial influx of Ca^{2+} into the dendrites of CA1 pyramidal neurons. The primary candidate for such a channel is the LVA or T-type Ca^{2+} channel (NS09482, NS11535, MH44754, and MH48432).

684.2

MECHANISMS OF ACTION POTENTIAL INITIATION IN SOMA, AXON HILLOCK, AND INITIAL SEGMENT OF PYRAMIDAL NEURONS. C. M. Colbert* and D. Johnston, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Pyramidal neuron dendrites propagate action potentials (APs), yet the site of AP initiation, even for dendritic input, appears to be the soma or axon hillock/initial segment (AH/IS) (Stuart & Sakmann, *Nature* 367:69, 1994). Recently, experimental findings have constrained the possible mechanisms underlying AP initiation, including an equal (and rather low) mean density of Na^+ channels in the soma and dendrites (Magee & Johnston, *J. Physiol.*, in press). Satisfying these constraints, models of pyramidal neurons predict that the AH/IS region must have a very high Na^+ channel density—perhaps two orders of magnitude greater than the soma. In such a configuration, the neuron cannot fire a robust spike without an IS. We tested these predictions using patch-clamp techniques to record from visually identified CA1 and subicular neurons and ISs in slices from adult rats. First, we estimated the density of Na^+ channels on the IS, AH, and soma using cell-attached patch recordings. The IS had only a slightly higher mean Na^+ channel density than the soma. Second, we assessed the ability of somatic and IS Na^+ channels to produce APs in the soma. Using whole-cell current-clamp recordings, APs were activated antidromically by alvear stimulation or orthodromically by depolarizing current to the soma. Tetrodotoxin (TTX) was puffed from a second pipette to block Na^+ channels. TTX applied to the IS (10–12 μm from the soma) reversibly blocked the antidromic AP in a graded fashion, while only slightly raising the threshold for the orthodromic AP. AP amplitude did not decrease significantly. TTX applied to the soma blocked somatic APs, although the much-attenuated antidromic AP still invaded the soma. These results suggest that the initial segment does not have a greatly increased Na^+ channel density and that somatic Na^+ channel activation is required to produce robust somatic action potentials (NS11535, MH44754, MH48432 and MH10896).

684.3

A STEADY-STATE, NIMODIPINE-SENSITIVE CALCIUM CURRENT ACTIVE AT REST IN HIPPOCAMPAL CA1 DENDRITES. D. Johnston*, J. C. Magee, B. R. Christie, and R. B. Avery, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The dendrites of hippocampal CA1 pyramidal neurons express a number of different types of voltage-gated channels, including Na^+ channels and several types of Ca^{2+} channels (Magee and Johnston, *J. Physiol.*, in press). The Ca^{2+} channels observed in dendrites include low-voltage activated (LVA) T-type channels, high-voltage activated (HVA) R-type channels, and dihydropyridine (DHP)-sensitive HVA L-type channels. During the investigation of LVA, Ni^{2+}-sensitive channels that are activated by EPSPs (Magee et al., this meeting), we found what appeared to be a standing Ca^{2+} current active at the resting potential.

Whole-cell recordings of visually identified CA1 neurons were made in hippocampal slices prepared from 5–8 week old rats. The pipettes were filled with 125 μM fura-2 for high-speed fluorescence imaging. When cells were hyperpolarized from rest, there was a time- and voltage-dependent decrease in $[Ca^{2+}]_i$ in the soma and apical dendrites. The decrease in $[Ca^{2+}]_i$ was maximal in the proximal dendrites and less in the soma and distal dendrites. A similar gradient was obtained when whole-cell recordings were made from dendrites. The voltage-dependent decrease in resting $[Ca^{2+}]_i$ was blocked by 200 μM Cd^{2+} and reduced by 73% with 10 μM nimodipine. Recent whole-cell voltage-clamp recordings from isolated pyramidal neurons have revealed a non-inactivating Ca^{2+} current activated near the resting potential (Avery and Johnston, this meeting). This Ca^{2+} current is partly sensitive to DHPs and thus may be involved in the decrease in $[Ca^{2+}]_i$ with hyperpolarization observed during the fluorescence imaging. The results suggest that a LVA, L-like Ca^{2+} current contributes to the resting properties of these neurons (MH44754, MH48432, NS11535, and NS09482).

684.4

AFTERHYPERPOLARIZATION IN NEONATAL PHRENIC MOTONEURONS: DIFFERENCES BETWEEN INSPIRATORY AND EXOGENOUSLY-EVOKED ACTION POTENTIALS. C. K. Su* & J. L. Feldman. Dept. of Physiol. Science, UCLA, LA, CA 90095-1527.

Repetitive firing behavior of phrenic motoneurons (PMNs) in neonatal rat were examined to elucidate the interplay between intrinsic neuronal properties and synaptic drive. Whole cell patch-clamp recordings were made from PMNs in *in vitro* brainstem-spinal cords of neonatal rat. Action potential afterhyperpolarizations were measured by averaging the waveform of action potentials. Extrinsic driving force was estimated from averages of synaptic potentials during inspiration. Maximal firing (intervals: 16 - 56 ms) typically occurred within the first 5 spikes of each inspiratory burst; this correlated with peak synaptic drive currents. Exogenous local application of phenylephrine (PE, 0.1-1 mM), an α_1 adrenergic agonist, did not significantly change the maximal firing rate, but increased average firing rate/cycle and lengthened the burst period; PE also induced tonic firing during the normally silent interbust period. Medium afterhyperpolarizations (mAHP) (duration~100 ms) were often observed following action potentials during expiration (elicited by PE), as well as following antidromic action potentials. However, mAHPs were infrequently observed following action potentials during inspiration. (Only some slow firing neurons showed a prominent mAHP during inspiration. For these neurons, mAHP was diminished by local application of PE.) Thus, mAHPs are seen following exogenously-but not endogenously-evoked action potentials. This suggests that intrinsic properties of PMNs are modulated by inspiratory synaptic factors to suppress mAHPs and modify the current-frequency response appropriate for inspiratory movement of the diaphragm. Supported by NIH Grant NS24742.

684.5

SPACIAL AND TEMPORAL INTEGRATION OF SYNAPTIC INPUT IN RAT CEREBELLAR PURKINJE CELLS *IN VITRO*

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We investigated spatial and temporal integration of synaptic input in cereballar Purkinje cells. Acute saggital slices (300 µm thick) were prepared from the cerebelli of two week old rats according to standard procedures. Somatic whole cell patch clamp recordings were established under visual control using an upright microscope with Normarski optics. The patch pipettes contained lucifer yellow to allow visualization of the individual Purkinje cells dendritic trees. Two small-diameter bipolar stimulating electrodes built from theta glass (tip diameter ~ 20 µm) were used to activate presynaptic fibers terminating on the recorded cells' dendrite. With the extension and geometry of the lucifer-yellow stained dendrite being well visible the stimulating electrodes were positioned above two remote sites of the dendritic tree such that synaptic input elicited by electrical stimulation would activate synapses on two independent branches of the dendrite. Synaptic currents and potentials elicited by the stimuli were measured under voltage clamp and current clamp conditions, respectively. Stimuli were applied through each of the electrodes seperately and the effect of each electrode was measured. Then pairs of stimuli with varying delays between the two stimuli were given in order to investigate temporal summation. Finally both electrodes were activated synchronously.

Under voltage clamp conditions somatically recorded excitatory synaptic currents summed up linearly. Preliminary results obtained under current clamp conditions suggest that excitatory synaptic potentials elicited at two independent dendritic branches also sum up linearly.

A Purkinje cell was anatomically reconstructed and a compartmental model of the cell was implemented on a computer. The experiments were repeated in the simulated cell.

684.6

ACTIVATION OF POSTSYNAPTIC CALCIUM/CALMODULIN PATHWAYS MODULATES SYNAPTIC RESPONSIVENESS IN HIPPOCAMPAL CA1 NEURONS. J-H. Wang* and P.T. Kelly, *Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas 77030.*

The role of calcium/calmodulin (Ca^{2+}/CaM) signaling pathways in synaptic transmission was studied in CA1 pyramidal neuron synapses made by Schaffer collateral/commisural terminals in rat hippocampal slices. The postsynaptic injection of a stoichiometric ratio of Ca^{2+}/CaM (4:1) induced significant potentiation of excitatory synaptic responses, EPSP (65±10%, mean±S.E.M, n=14) and EPSC (54±7%, n=10), even in the presence of $GABA_A$ and $GABA_B$ antagonists (79±9%, n=8). Potentiation was not induced by injecting Ca^{2+} (4±4%, n=12) or CaM alone (10±7%, n=12) and was blocked by co-injection of a CaM-binding peptide (CBP). Reciprocal experiments showed that Ca^{2+}/CaM-induced synaptic potentiation and tetanus-induced LTP occluded one another. Pseudosubstrate inhibitors or high-affinity substrates of CaM-KII ($[Ala^{286}]CaMKII_{281-302}$ and autocamtide-3) or PKC (PKC_{19-31} and $neurogranin_{28-43}$) significantly blocked Ca^{2+}/CaM-induced potentiation. These results indicate that postsynaptic levels of free Ca^{2+}/CaM are a rate limiting factor for triggering synaptic potentiation and that functional cross-talk between Ca^{2+}/CaM and PKC pathways occurs during LTP induction.

Paired-pulse facilitation (PPF) of synaptic transmission was significantly attenuated by Ca^{2+}/CaM injections under current-clamp (37±5% reduction, n=10) and voltage-clamp conditions (31±5%, n=7), and in the presence of GABAergic antagonists (43±11%, n=8). The attenuation of PPF was blocked by CBP, psuedosubstrates and substrates of CaM-KII or PKC, and reversed by cyclothiazide, a blocker of AMPA receptor desensitization. These results indicate that PPF is regulated by postsynaptic Ca^{2+}/CaM pathways and AMPA receptor plasticity. (supported by NIH grant NS32470).

684.7

COINCIDENCE DETECTION OF SYNAPTIC INPUTS AND SPIKES BY INDIVIDUAL SPINES REVEALED BY CALCIUM IMAGING WITH TWO-PHOTON MICROSCOPY.

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Most synaptic connections in the central nervous system occur on dendritic spines, whose functional properties are poorly understood because of experimental difficulties resulting from their small sizes (~1 μm^3). We have taken advantage of the improved tissue penetration provided by two-photon excitation of visible fluorophores with infrared light (Denk et al., Science 248: 73-76, 1990) to image calcium concentration in spines from CA1 pyramidal neurons in slices of rat hippocampus. Cells were filled with the calcium indicators Calcium Green 1 or 5N, using whole-cell perfusion. Laser scanning was performed using a modified confocal microscope with a mode locked Ti:Saphire laser (λ=850 nm) as the excitation source.

Focal subthreshold synaptic stimulation produced calcium accumulations that were restricted to isolated spines, showed stochastic failures, and were abolished by postsynaptic blockers. Similarly, discrete calcium accumulation localized to isolated spines were seen in spontaneous calcium accumulations elicited by high [Ca^{2+}] and low [Mg^{2+}] ACSF, conditions that enhance spontaneous transmitter release. In contrast, single somatic spikes induced fast-peaking calcium accumulations in spines throughout the cell. With 2 msec time resolution, we observed no appreciable delay between spike-induced calcium accumulation in spines heads and nearby dendrites. Finally, the simultaneous pairing of spikes with synaptic stimulation was frequently cooperative resulting in calcium accumulations that were supralinear, i.e. larger than the combined responses to the individual stimuli.

Taken together, our results strongly suggest: (i) the existence of voltage-sensitive calcium channels in the spine heads, (ii) their antidromic activation by the action potential, (iii) that individual spines are calcium compartments and (iv) that they can individually signal the coincidence of the input and output of the neuron, thus serving as basic functional units of synaptic integration.

(Supported by the Office of Naval Research and AT&T Bell Laboratories)

684.8

DENDRITIC CALCIUM-DEPENDENT EXOCYTOSIS IN CULTURED HIPPOCAMPAL NEURONS: ROLE OF CALCIUM/CALMODULIN PROTEIN KINASE II. M. M. Maletic-Savatic*, T. Koothan and R. Malinow, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA

Calcium/calmodulin protein kinase II (CaMKII) has been implicated as a key enzyme involved in formation of long term potentiation (LTP). One of the possible models of LTP formation is that CaMKII activation mediates insertion of glutamate receptors into postsynaptic membrane by vesicle exocytosis. In order to test this hypothesis we have used quantitative time-lapse imaging of the fluorescent membrane probe FM 1-43. Exocytosis of vesicles in cultured hippocampal neurons was monitored at 4 to 10 days *in vitro* (DIV). Neurons exposed overnight to FM 1-43 accumulate dye in all neuronal processes, some of which are dendrites. Release of the dye from dendritic sites was induced by the Ca++ ionophore A23187 (2.5 μM). This release was observed at ages (DIV) when CaMKII and glutamate receptors (NMDAR1, GluR1, GluR2/3 and GluR4) are normally expressed, but not before. We investigated the pattern of FM1-43 release at earlier ages, following infection of the cultured neurons with a recombinant vaccinia virus expressing a full length (f) CaMKII. Preliminary results indicate that the presence of fCaMKII influences the release of the FM1-43 dye by A23187 at an age when NMDAR1 is expressed but CaMKII and GluRs are normally not expressed. These results suggest a role for CaMKII activation in exocytosis at postsynaptic sites.

684.9

AT-SOURCE KINETICS OF $GABA_A$ RECEPTOR MEDIATED IPSCs IN DENTATE GYRUS GRANULE CELLS. I. Soltesz,*[1] D.K. Smetters[2] and J. Mody[3]. [1]Dept. of Anatomy & Neurobiol., UC Irvine, CA; [2]Salk Institute, La Jolla, CA; [3]Dept. of Neurology, UCLA, Los Angeles, CA.

Principal cells receive their GABAergic inputs from multiple interneuron types which terminate in mutually exclusive domains along the their longitudinal axis. In the dentate gyrus both somatic and dendritic layers contain several $GABA_A$ receptor subunits and an abundance of GABAergic terminals. We used whole-cell patch clamp and computational techniques to find out whether there are differences in the properties of inhibitory synapses located on different parts of cells. IPSCs were recorded from adult rat granule cells in the presence of 10 μM CNQX and 40 μM D-AP5, with 130 mM CsCl, 10 mM HEPES, and 2 mM $MgCl_2$ containing electrodes.

Minimal stimulation at various distances from the somatic recording site showed that both proximal and distal inhibitory fibers are capable of releasing GABA, and that the released transmitter activates functional $GABA_A$ receptors. In addition, these experiments demonstrated that the further away from the soma the IPSCs were generated, the slower and smaller they became. When spontaneous events were examined in the presence of TTX (miniature IPSC or mIPSCs), the 10-90% rise times of the events ranged from 0.1 to 10 ms. How are mIPSCs with such different kinetics generated? Compartmental modeling of a typical granule cell showed that the kinetics of distal minimally evoked IPSCs at source are comparable to the kinetics of the somatic mIPSCs. This suggests that activation of somatic and dendritic $GABA_A$ receptors evokes events with very similar at-source kinetics. However, when the bulk (>50%) of the dendritic tree was removed, the distribution of 10-90% rise times still showed an unchanged low (<3%) percentage of events with extremely slow kinetics (3-10ms), indicating that such slow events can be generated at proximal sites.

These findings suggest that in granule cells most distal and proximal synapses contain $GABA_A$ receptors with similar kinetics, however, they also indicate the possible presence of a small number of synapses with distinct $GABA_A$ receptor composition and kinetics.

684.10

RECOMBINANT GEPHYRIN FORMS FILAMENTS *IN VIVO* WHICH BIND THE GLYCINE RECEPTOR β SUBUNIT AND $GABA_A$ RECEPTOR β3 SUBUNIT J. Kirsch*, and H. Betz Dept. of Neurochemistry, Max-Planck-Institute for Brain Research, Deutschordenstr. 46, 60528 Frankfurt, Germany

The peripheral membrane protein gephyrin was originally identified upon co-purification with the inhibitory glycine receptor of rat spinal cord. Expression of this polypeptide in spinal neurons is essential for the formation of postsynaptic glycine receptor clusters. Gephyrin is widely expressed throughout the synaptic regions of the central nervous system and co-distribution with glycine- and $GABA_A$ receptors could be demonstrated. Since both receptor types are pentameric receptor complexes composed of several subunits we investigated by heterologous expression in combination with electron microscopy and immunocytochemistry which receptor subunits can interact with recombinant gephyrin. We found that recombinant gephyrin forms filamentous structures (6nm) in HEK 293 cells which bind glycine- or $GABA_A$ receptor β subunits. Therefore, we conclude that gephyrin-mediated anchoring at synaptic sites may depend on the subunit composition of the respective neurotransmitter receptors.

684.11

1S,3R-ACPD INCREASES INTRACELLULAR CALCIUM LEVELS IN RAT DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS. F.Zheng* and J.A. Connor, Roche Inst. of Molecular Biology, Nutley, NJ 07110.

1S,3R-ACPD causes membrane depolarization and burst firing in rat DLSN neurons, and results in long lasting enhancement of synaptic transimission (Zheng & Gallagher, Neuron 9, 163-172, 1992). One possible mode of action is an increase of intracellular calcium levels by releasing calcium from IP3-sensitive stores due to activation of a metabotropic receptor coupled to phospholipase C. In the present study, we measured intracelluair calcium levels using fluorescent imaging techniques in a slice preparation. Fura-2 was injected into DLSN neurons through the recording microeletrode. $[Ca^{2+}]_i$ was measured by 350/380 ratio.

Superfusion of 1S,3R-ACPD depolarized the fura-2 loaded DLSN neurons and triggered burst firing as described previously. No significant change of $[Ca^{2+}]_i$ was associated with the simple depolarization, but a large rise of $[Ca^{2+}]_i$ occured after burst firing started. Calcium increases first occurred in remote dendrites, then spread gradually into the soma. During the repolarizing phase of the burst firing, the dendritic calcium signals returned to basal level first, while the calcium level in the soma was still elevated. $[Ca^{2+}]_i$ changes during ACPD bursts were much larger than those produced by spike activity in normal saline. Since the enhancement of spike-triggered calcium increases by 1S,3R-ACPD is accompanied by a prolonged, depolarizing plateu potential which is thought to be a dendritic calcium spike, this action of 1S,3R-ACPD is likely due to an enhancement of calcium influx through voltage-gated calcium channels. However, the $[Ca^{2+}]_i$ change during bursts was reduced by pretreating slice with thapsigargin (5 μM), suggesting at least part of calcium increase is due to release from internal stores. Thapsigargin has no consistant effects on basal calcium level. On the other hand, ryanodine (20-100 μM) did not reduce, but enhanced the ACPD-induced calcium increase associated with bursting. Our data suggested that the rise of $[Ca^{2+}]_i$ associated with ACPD-induced bursting is due to both calcium influx and calcium release from internal stores. (Supported in part by Jeane E. Kempner Scholarship to F.Z.)

684.12

TIME COURSE OF EXCITATORY SYNAPTIC TRANSMISSION IN THE NUCLEUS TRACTUS SOLITARIUS FROM RAT

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Brain stem neurones in the nucleus tractus solitarius (NTS) from rats are well suited for patch clamp analysis of synaptic currents as their passive electrical properties are characterized by a single membrane time constant of 0.2ms and an input resistance above 1GOhm. Glutamate receptor mediated synaptic transmission was investigated in parvocellular NTS neurones by performing patch clamp recordings in thin slices from 10 - 14 day old rats. Stimulation of visceroafferent fibers evoked AMPA receptor mediated EPSCs which were characterized by fast activation times of 0.5 ±0.2 ms (20 - 80 % max. ampl., n = 5) and slow decay time constants of 4.0 ± 0.6 ms (n = 5). Slow decay kinetics were also found for spontaneous and miniture EPSCs investigated in 1 μM TTX. The slowness of decay did not result from dendritic filtering. Also, it did not reflect the deactivation kinetics of the postsynaptic AMPA receptors, which were investigated by rapid (< 0.3 ms) application of glutamate to outside - out patches pulled from the same cells. Comparison with fast EPSC decays in other systems suggests that the slow decay in NTS neurones results from the prolonged presence of glutamate during the synaptic transmission process.

685.1

A NOVEL PROTEIN (HAP-1) ENRICHED IN BRAIN INTERACTS WITH THE HUNTINGTON'S DISEASE PROTEIN
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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expanding glutamine repeat in a gene termed IT 15 or Huntingtin, which has no significant homology to other known genes. The neuropathology of HD is restricted to the brain. However, Huntingtin mRNA and its protein product are widely expressed in brain and in the periphery, with no clear difference in level of expression between patients and controls. Both the genetics of the disorder and the expression of the expanded allele of the protein in HD patients suggest that the mutation induces a toxic gain of function, possibly dependent on interactions of the mutated protein with other cellular proteins. Using the yeast two hybrid system, we have now identified a cDNA encoding a novel protein (Huntingtin Associated Protein or HAP-1) which interacts with the HD protein. In vitro binding assays with GST fusion proteins and immunoprecipitation experiments confirm the interaction and show that the association is enhanced by an expanded polyglutamine repeat in the HD protein. Northern and Western blot analyses demonstrate that the HAP-1 gene product is highly expressed in brain, and thus may be involved in the selective brain pathophysiology of HD.

685.2

CELLULAR LOCALISATION OF THE HUNTINGTON'S DISEASE PROTEIN AND DISCRIMINATION OF THE NORMAL AND MUTATED PROTEIN.
Frédéric Saudou[1*], Yvon Trottier[1], Didier Devys[1], Georges Imbert[1], Isabelle An[2], Chantal Weber[1], Yves Agid[2], Etienne C. Hirsch[2] and Jean-Louis Mandel[1]. [1]IGBMC, 67404 Illkirch Cedex, France and [2]INSERM U289, 75651 Paris, France.

Huntington's disease (HD) is a dominant neurodegenerative disorder, characterised by involuntary movements (chorea), personality changes and dementia. It is caused by an expansion of a polyglutamine coding CAG repeat in a gene of unknown function. The wide expression of the transcript does not correlate with the pattern of neuropathology in HD (neuronal death in the striatum). To study the HD gene product (huntingtin), we have developed monoclonal antibodies raised against four regions of the protein. These monoclonals detect specifically huntingtin as a ~350 kDa protein in various human cell lines and in neural and non-neural rodent tissues. In cell lines from HD patients a doublet protein is detected corresponding to the mutated and normal huntingtin, while in post-mortem brains from 3 HD patients the mutated huntingtin was barely detectable suggesting that the abnormal protein may be less stable in brain. Immunohistochemical studies in the human brain using two of these mAbs detect the huntingtin in perikarya of some neurons, in neuropiles, varicosities and as punctate staining likely to be nerve endings. This localisation in nerve endings suggest that huntingtin may play a role in neurotransmission. These monoclonal antibodies are being used to identify proteins that interact with huntingtin and to analyse the relative accumulation of the normal and mutated protein in brain. We have generated neural cell lines that express high amounts of mutated huntingtin, and transgenic mice carrying a mutated HD cDNA (73 repeats) have been obtained.

685.3

TARGETED DISRUPTION OF THE MURINE HUNTINGTON'S DISEASE GENE I: EARLY POST-IMPLANTATION EMBRYONIC LETHALITY IN HOMOZYGOTES.
J. Nasir[1,4], V. M. Diewert[2], J. M. Richman[2], J. Zeisler[1,4], M. von Krosigk[3], and Michael R. Hayden[1,4] Depts. of [1]Medical Genetics,[2] Clinical Dental Sciences,[3] Psychiatry and [4]Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, B.C., Canada.

Huntington's Disease (HD) is a devastating adult-onset neurological disease associated with chorea, cognitive deficit, psychiatric disturbances and inexorable progression to death, typically 10 to 15 years after onset. It is associated with expansion of CAG repeats within the coding region of a novel gene, which is widely expressed but causes selective neuronal death. A similar mutational mechanism with expansions of CAG repeats is associated with five other neurodegenerative diseases.. In addition, the question of how these widely expressed genes cause selective neuronal death remains unanswered. To understand the physiological function of the HD gene and its role in development, we have created a targeted disruption in exon 5 of *Hdh* (*Hdh* ^{ex5}), the murine homologue of the HD gene and characterised both homozygotes and heterozygotes (see accompanying poster) for this mutation. No homozygous animals were found among 225 liveborns derived from an intercross between heterozygous animals. However, an analysis of timed matings showed a high frequency (22%) of embryos were being resorbed between day 7.5 and 9.5 of development. The majority of resorbed embryos (80%) were homozygous for the *Hdh* ^{ex5} mutation. These mutant embryos initiate gastrulation and form the three germ layers (ectoderm, endoderm and mesoderm) but there is subsequent failure to neuralate. Consequently, the embryos fail to develop a normal anterior-posterior axis. In addition, they are unable to form somites and do not proceed to organogenesis. Therefore, we propose the HD gene is essential for post-implantation embryonic development.

685.4

TARGETED DISRUPTION OF THE MURINE HUNTINGTON'S DISEASE GENE II: BEHAVIORAL AND NEUROPATHALOGICAL ASSESMENT IN HETEROZYGOTES
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In this second series of experiments investigating the function of the *Hdh* gene, we assessed the behavioral and neuropathalogical effects of heterozygous deletion of the *Hdh* gene (*Hdh*^{Ex5}). Seven *Hdh*^{Ex5} heterozygous mice and 7 normal littermates were subjected to a battery of 4 behavioral tasks. Heterozygous mice were (1) hyperactive relative to controls during a standard locomotion test (total photocell counts: heterozygotes=3545 ± 275; wildtype=2272 ± 275, $p<0.01$); (2) showed no difference from controls on performance on either a T-maze task, (which assessed spontaneous alternation behavior), or (3) a radial-arm-maze task (which assessed working memory). On the Morris Water Maze (4), a task used to assess spatial navigation abilities, heterozygous mice showed similar learning to control animals. However, when the location of the hidden platform was switched to another area of the maze, heterozygous mice had longer latencies to find the hidden platform relative to control mice (mean latency/trial; heterozygotes=66.3 ±11s , wildtype= 30.2 ± 11s, $p<0.05$) indicating a lack of behavioral flexibility. Subsequent neuropathological analysis was performed on 2 *Hdh*^{Ex5} mice and 2 controls used in the above experiments. Heterozygous mice showed ≈43% cell loss in the globus pallidus ($p=0.051$) and ≈45% cell loss in the subthalamic nuclei ($p=0.021$) relative to control mice. These data suggest that the *Hdh* gene may play an important role in the normal functioning of the basal ganglia, and that heterozygous mice show some behavioral and morphological abnormalities with similarity to those seen in patients with Huntington's Disease.

685.5

CHARACTERIZATION OF THE DRPLA DISEASE GENE PROTEIN PRODUCT (ATROPHIN-1): COMPARISON WITH HUNTINGTON'S DISEASE GENE PRODUCT (HUNTINGTIN)
A.H. Sharp*, S.H. Li, G. Schilling, J. Bao, T.M. Dawson C.A. Ross. Laboratory of Molecular Neurobiology, Johns Hopkins University, School of Medicine, Baltimore, MD 21205-2196.

Dentatorubral pallidoluysian atrophy (DRPLA or Smith's Disease), like Huntingtons's Disease (HD), is an autosomal dominant neurodegenerative disease. Both diseases are caused by expansion of CAG repeats within the coding regions of their respective genes. Although the genes share no homology to each other or to other genes, the two diseases share many clinical, genetic and pathological features. Both diseases are thought to involve gain of function mutations suggesting effects at the protein level. We are using specific antibodies developed against the protein products of the two genes to compare their cellular and subcellular distributions and biochemical properties. Both proteins have widespread distributions in brain and other tissues. Immunohistochemical experiments show that atrophin-1, like huntingtin, is neuronal with a cytoplasmic distribution. Subcellular fractionation of rat brain tissue indicates that both proteins are present in both soluble and particulate fractions. Both are enriched in a high speed pellet prepared from an osmotically lysed synaptosome fraction (LP2). Sucrose gradient centrifugation of soluble extracts from brain tissue show that both proteins in their native forms migrate with higher apparent molecular weights than expected for monomers, suggesting that both dimerize or form complexes with other proteins.

685.6

TOWARD AN UNDERSTANDING OF TARDIVE DYSKINESIA: CHRONIC HALOPERIDOL ENHANCES NMDA TOXICITY IN MOUSE STRIATUM.
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Chronic treatment with neuroleptic drugs leads to the development of tardive dyskinesia (TD) in a large proportion of patients. The supersensitivity of dopaminergic receptors explains neither the onset nor the persistence of TD. The nature and the location of mechanisms in the basal ganglia that are responsible for the development of TD and irreversibility of the symptoms remain unresolved issues. In patients subjected to chronic treatment with neuroleptic drugs who develop TD concentration of glutamate is increased in CSF. Therefore we examined whether acute or chronic systemic treatment with haloperidol (1-10 mg/kg, i.p. for 21 d) modulates toxicity of glutamate and N-methyl-D-aspartate (NMDA) in the mouse striatum. Glutamate (1 µmol) and NMDA (1 nmol) were microinjected into the striatum of NMRI mice, 20-25 g, subjected to acute or chronic treatment with haloperidol, and stereologic analysis of the damage was performed 24 h later. In mice subjected to single systemic administration of haloperidol, non-toxic dose of either glutamate or NMDA induced no damage in the striatum, whereas in mice subjected to chronic treatment with haloperidol (5 and 10 mg/kg/d for 21 d) both glutamate and NMDA produced pronounced excitotoxic lesions in the striatum in a dose-dependent manner. These data indicate that long-term treatment with haloperidol increases susceptibility of striatum to excitotoxic action of glutamate and NMDA. Such observations form the base for the hypothesis that chronic treatment with neuroleptic drugs may change the balance between neurotransmitters in the basal ganglia in such a way that even physiological (non-toxic) concentrations of glutamate may become neurotoxic and acting via NMDA receptors lead to neuronal damage in the mouse striatum. The hypothesis of a weak glutamergic toxicity after termination of long-term treatment with neuroleptic drugs offers alternative explanation for the evolution and persistence of the symptoms of TD.

685.7

NEUROPROTECTIVE STRATEGIES FOR TREATMENT OF LESIONS PRODUCED BY MITOCHONDRIAL TOXINS: IMPLICATIONS FOR NEURODEGENERATIVE DISEASES. M. F. Beal*, M.T. Matthews, D.R. Henshaw and J.B. Schulz. Neurochemistry Laboratory, Neurology Service, Massachusetts General Hospital, Boston, MA 02114.

Neuronal death in neurodegenerative diseases may involve energy impairment leading to secondary excitotoxicity, and free radical generation. Potential therapies for the treatment of neurodegenerative diseases therefore include glutamate release blockers, excitatory amino acid receptor antagonists, agents to improve mitochondrial function and free radical scavengers. In the present study we examined whether these strategies either alone or in combination had neuroprotective effects against striatal lesions produced by mitochondrial toxins. The glutamate release blockers lamotrigine and BW1003C87 significantly attenuated lesions produced by intrastriatal administration of 1-methyl-4-phenylpyridinium (MPP^+). Lamotrigine significantly attenuated lesions produced by systemic administration of 3-nitropropionic acid (3-NP). Memantine, an N-methyl-D-aspartate (NMDA) antagonist, protected against malonate induced striatal lesions. We previously found that coenzyme Q_{10} and nicotinamide, and the free radical spin trap n-tert-butyl-α-(2-sulfophenyl)-nitrone (S-PBN) dose-dependently protect against lesions produced by intrastriatal injection of malonate. In the present study we found that the combination of either lamotrigine or MK-801 with coenzyme Q_{10} exerted additive neuroprotective effects against malonate. Lastly the combination of nicotinamide with S-PBN was more effective than either agent alone. These results provide further evidence that glutamate release inhibitors and NMDA antagonists can protect against secondary excitotoxic lesions in vivo. Furthermore they show that combinations of agents which act at sequential steps in the neurodegenerative process can produce additive neuroprotective effects.

685.8

EXCITOTOXIC DEGENERATION OF MOTOR NEURONS RESEMBLES THAT SEEN IN SOD-1 TRANSGENIC MICE. J.W. Olney*, C. Ikonomidou, G.J. Wang, Y.Q. Qin and M.T. Price; Washington Univ. Med. Sch, St. Louis, MO.

Following the discovery that patients with familial amyotrophic lateral sclerosis have a gene defect at a locus that encodes superoxide dysmutase 1 (SOD-1), several groups have developed SOD1 transgenic mice and have observed that these mice at 5 to 8 wks of age display spontaneous onset of paralysis and motor neuron degeneration, which involves vacuole formation in cell bodies and processes and swelling of the proximal axonal segment. Because other lines of evidence have implicated an excitotoxic mechanism in motor neuron degeneration in ALS, the question arises whether the morphological changes described in the SOD mutant mice are consistent with an excitotoxic process. To address this question, we have undertaken studies aimed at providing an accurate description of excitotoxic degeneration of spinal motor neurons. The excitatory amino acid agonists, DL-homocysteic acid (DL-HCA) or kainic acid (KA), when given systemically to infant rats or when applied directly to the exposed lumbar cord following posterior laminectomy of 21 day old rats, cause excitotoxic degeneration of motor neurons. DL-HCA is a mixed agonist that acts at both NMDA and non-NMDA receptors whereas KA acts only at non-NMDA receptors. The pathological changes in motor neurons following either DL-HCA or KA treatment were studied by both light and electron microscopy and consisted of intracytoplasmic vacuole formation and dark cell changes in which the entire motor neuron, except the vacuoles, gradually became condensed and shrunken. These changes occurred within the first 2 hrs after exposure to the excitotoxin and were accompanied by changes in the proximal axonal segment consisting of swelling of the proximal myelin sheath with accompanying changes in axoplasmic constituents. Degenerative changes in more distal parts of the axons developed over a 12-24 hr period. We conclude that on the basis of purely morphological criteria, the spontaneous degenerative process affecting motor neurons of SOD-1 transgenic mice is consistent in appearance and time course with degeneration induced by exposure to excitotoxin agonists. Supported by AG 11355 and RSA MH 38894 (JWO).

685.9

BCL-2 GENE THERAPY IN TRANSGENIC MICE EXPRESSING HUMAN SUPEROXIDE DISMUTASE I WITH A MUTATION LINKED TO FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS. D.R. Borchelt*[1], P.C. Wong[1], C.A. Pardo[1], J. Rothstein[2], N.G. Copeland[3], N.A. Jenkins[3], S.J. Korsmeyer[4], D.W. Cleveland[5], D.L. Price[1,2,6], and D. Merry[7]. Dept. of [1]Pathology, [2]Neurology, and [6]Neuroscience, The Johns Hopkins Univ. School of Medicine, Balt., MD 21205; [3]National Cancer Institute, Frederick, MD; [4]Washington Univ., St. Louis, MO.; [5]Ludwig Inst. for Cancer Research and Dept. of Neuroscience, Univ. of Calif. San Diego, CA 92093; [7]Univ. of Pennsylvania, Philadelphia, PA.

We have generated several lines of transgenic mice expressing human (Hu) superoxide dismutase I (SOD1) with a mutation (glycine 37 to arginine - G37R) that causes familial amyotrophic lateral sclerosis (FALS). These animals develop motor neuron disease; the age of onset, duration, and age at death are strongly dependent upon the dose of the mutant protein. Several investigations have shown that expression of the protooncogene Bcl-2 can protect neural cells from a variety of insults. To determine whether Bcl-2 is protective against the injury caused by mutant SOD1, transgenic mice expressing the G37R mutant were mated to mice expressing human Bcl-2 driven by the human neurofilament-L (NF-L) promoter; HuBcl-2 accumulates to 5-10 times the level of endogenous mouse Bcl-2 in these mice. In the lines of mice expressing the highest level of mutant SOD1 [G37R(42)], little if any delay in the age of onset of disease was observed in the presence of elevated human Bcl-2. We are presently examining additional parameters, including the duration of the disease and the pathological appearance of affected neurons. Whether Bcl-2 will alter the age of onset or duration of disease in animals with a lower dose of mutant SOD1 is also being examined.

685.10

A SPECIFIC HUMORAL RESPONSE TO A NOVEL OLIGODENDROCYTE-SPECIFIC PROTEIN IN PATIENTS WITH RELAPSING MULTIPLE SCLEROSIS. Bronstein JM*, Seitz RS, Lallone R, Harrington WT, Rosenblatt J, Ellison GW, Myers LW,. Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024, Research Genetics, Birmingham, AL 35209, and U of Miami School of Medine, FL 33101.

We have recently described a novel oligodendrocyte-specific protein (OSP) found only in CNS myelin. To determine if there is a humoral response to OSP in patients with multiple sclerosis (MS), we performed Western blot analysis and enzyme-linked immunoabsorbent assay (ELISA) on CSF samples. Using human brain homogenate and recombinant mouse OSP as antigens, Western blots demonstrated anti-OSP antibodies in 6 of 6 CSF samples of relapsing remitting MS patients. Peptide mapping of the entire protein was performed and 2 peptides were identified that reacted strongly with pooled CSF of MS and control patients. One of these peptides was used for ELISA to determine anti-OSP titers. Twenty-four of 30 (80%) of CSF samples from relapsing remitting MS patients (stable for at least 1 month) had ELISA optical density units of > 0.55 (mean ± SD, 0.74 ±.35). Zero of 8 CSF samples from HTLV-I associated myelopathy (HAM) patients (0.29 ± .17) and 0 of 9 normal controls (0.33 ± .09) had values above 0.55 units. In CSF from chronic progressive MS (CP) patients, 2 of 10 samples had values above 0.55 units (0.39 ±.32). No antibody reaction was observed using several other peptides as antigens. These data demonstrate that anti-OSP antibodies are present in significant titers in CSF of the majority of relapsing MS patients, fewer CP patients, and none of the normal or HAM control samples. Further investigation is necessary to determine the role these antibodies play in the pathogenisis of MS or their usefullness as a diagnostic test.

685.11

POTENTIAL INVOLVEMENT OF THE PROTEIN KINASE C PATHWAY IN HIV-1 ASSOCIATED NERVOUS SYSTEM ALTERATIONS. T. Wyss-Coray*, E. Masliah, S. M. Toggas, H. S. Lee and L. Mucke. Dep. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037, and Depts. of Neurosciences and Pathology, UCSD, La Jolla, CA 92093-0624

HIV-1 associated central nervous system (CNS) disease involves neuronal damage and prominent reactive astrocytosis, the latter being characterized by strong upregulation of the glial fibrillary acidic protein (GFAP) in astrocytes. Similar alterations are found in transgenic (tg) mice expressing the HIV-1 envelope protein gp120 in the CNS (Toggas et al., Nature 367:188-193). Because alterations of astrocyte functions could contribute to neuronal impairment we compared brains of gp120 tg mice and gp120-transfected C6 astrocytoma cells with controls, and found that gp120 induced a prominent elevation of steady state GFAP mRNA levels, due primarily to transcript stabilization. Increased levels of GFAP mRNA were also induced in non-transfected C6 cells by exposure to recombinant gp120. Gp120 effects were significantly decreased by inhibitors of protein kinase C (PKC) but not by inhibitors of protein kinase A. PKC activity was upmodulated in gp120 transfected C6 cells and in the CNS of gp120 transgenic mice. Further, brain tissue from patients with HIV-1 encephalitis and from gp120 transgenic mice showed increased PKC immunoreactivity. Taken together, these results indicate that gp120-induced increases in PKC activity may contribute to the gliosis seen in gp120 transgenic mice as well as in HIV-1 infected humans and raise the question of whether dysregulation of signal transduction pathways represents a general mechanism of HIV-associated pathogenesis.

685.12

NEURONAL CELL DEATH IN PRION DISEASE

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Neuronal degeneration and cell death are among the most important features of prion diseases, the mechanisms of which are only poorly understood. Our studies so far have been focused on two questions. First, what mechanisms are involved in neurotoxicity of the prion protein (PrP) or synthetic peptides thereof? Second, is cell death in prion diseases mediated by mechanisms of apoptosis? Neuronal cell death in prion diseases was studied in three experimental systems.

1. DNA fragmentation and morphology of cell death in mice infected with the scrapie strain 79A.
2. The effect of PrP 106-126, a synthetic peptide corresponding to amino acids 106 to 126 of PrP, on cultures of cortical cells from embryonic day 16 mice.
3. The effect of PrP 106-126 on cultures of cortical cells from embryonic day 16 $PrP^{0/0}$ mice (PrP knock-out mice).

Cell death in the 79A scrapie strain in C57 mice was studied using in situ end labeling (ISEL), and light and electron microscopy. Our results show that apoptosis occurs in an experimental scrapie system in vivo. In this system apoptosis is observed particularly in the outer nuclear layer of the retina and the granule cell layer of the cerebellum. In cell culture experiments we showed that PrP 106-126 is toxic to cortical cells from embryonic day 16 mice. PrP 106-126 has no neurotoxic effect on cortical cells from knock-out mice. This is the first difference between neurons from normal and from knock-out mice observed by us, since using patch-clamp analysis we were unable to confirm differences in the synaptic transmission beween normal and knock-out neurons in the cerebellum. In addition, our results show that the neurotoxicity of PrP 106-126 can be blocked with MK801 and verapamil indicating that it is mediated by intracellular Ca^{++} increase.

686.1

SENSORY TRANSDUCTION IN THE VOMERONASAL ORGAN: IONIC CURRENTS OF DISSOCIATED MOUSE RECEPTOR NEURONS. E. R. Liman and D. P. Corey*. Howard Hughes Medical Institute and Massachusetts General Hospital, Boston MA, 02114.

We are investigating the mechanism of sensory transduction in the vomeronasal organ (VNO). The VNO is located at the base of the rostral portion of the nasal cavity and contains chemosensory neurons that are thought to respond to pheromones. While signaling pathways have been elucidated for olfactory transduction, they have not been for the VNO. There are several reasons to believe that transduction may be different in the two systems. For example, cilia- the site of transduction in olfactory neurons- are not present on VNO neurons; VNO neurons instead bear microvilli.

We have developed a protocol for preparing dissociated mouse VNO neurons that yields high numbers of relatively intact cells. The diameter of the VNO neuron cell body is 11.2 ± 0.4 μm (n=12), which is considerably larger than that of olfactory neurons (5-8 μm). A dendritic process is evident that ranges in length from a 2 to more than 30 μm and ends in a tuft of microvilli.

As a first step towards understanding transduction, we are using whole cell patch clamp recording to characterize voltage-activated conductances. In experiments with Cs^+ in the pipette, inward Na^+ currents were first evident with voltage steps to -50 mV and reached a peak at ~ +10 mV. The currents were half inactivated by a prepulse to -67 ± 3 mV (n=5), similar to rat olfactory Na^+ currents. TTX, at a concentration of 300 nM, blocked 54 ± 9 % (n=5) of the current. We have also recorded K^+ and Ca^{++} currents and are in the process of characterizing them.

We have begun to investigate whether VNO neurons express cyclic-nucleotide-gated channels. We have found no positive evidence for the presence of cyclic nucleotide gated channels either by excised patch recording or intracellular dialysis with cyclic nucleotides and are in the process of confirming these results.

686.2

GLUCOCORTICOID (TYPE II) RECEPTORS IN THE MAMMALIAN OLFACTORY MUCOSA: RU 28362 BINDING SITES. R.C. Kern[1], J.D. Foster[2] and D.Z. Pitovski[1]* [1]Dept. of Otolaryngology - Head and Neck Surgery, Northwestern University, Chicago, IL 60201, [2]Dept. of Anatomy and Cell Biology, Meharry Medical College, Nashville, TN 37208.

The synthetic corticosteroids are the most frequently prescribed medications for olfactory disorders despite the fact that their mechanism of action within the olfactory system is unknown. Recently, a series of studies was initiated to gain insight into the mechanism of coticosteroid action on the olfactory mucosa. In this current study, we have focused on the binding characteristics of the glucocorticoid (type II) receptor.

The synthetic glucocorticoid ^{3}H-RU 28362, which has negligible binding to mineralocorticoid (type I) receptors (Philibert et al., *Annu. Meet. Endocrine Soc. 65th, 1983, p 335*), was employed to identify high-affinity glucocorticoid (type II) receptors in the olfactory mucosa. By Scatchard plot analysis, the K_d of the [^{3}H]-RU 28362-cytoplasmic receptor complex was 2.2 x 10^{-9} M, while the concentration of binding sites, B_{max}, was 180 fmol/mg dry tissue. Time course studies indicated that the binding of [^{3}H]-RU 28362 by the olfactory tissues reached equilibrium within 30 min of incubation at 25°C.

Substantial specific [^{3}H]-RU 28362 binding to the olfactory tissues suggests the presence of glucocorticoid receptors and sites of glucocorticoid action in the olfactory mucosa.

(Supported by Northwestern University Research Fund and NIH CIDA DC00046)

686.3

IMMUNOHISTOCHEMICAL LOCALIZATION OF GLUCOCORTICOID (TYPE II) RECEPTOR IN THE MAMMALIAN OLFACTORY MUCOSA. J.D. Foster[1]*, R.C. Kern[2], and D.Z. Pitovski[2]. [1]Dept. of Anatomy and Cell Biology, Meharry Medical College, Nashville, TN 37208, [2]Dept. of Otolaryngology - Head and Neck Surgery, Northwestern University Medical School, Chicago, IL 60201.

Immunohistochemical localization of glucocorticoid (type II) receptor in the mammalian olfactory mucosa was examined by utilization of an affinity-purified antibody raised against a glucocorticoid receptor protein. Immunoreactivity was associated with the acinar cells of Bowman's glands located in the lamina propria. In addition, the olfactory nerve axons were also immunoreactive. Within the olfactory epithelium, immunoreactivity for this receptor was also observed, which by location corresponds to sustentacular cells and/or olfactory receptor cells. There was no immunocytochemical staining when the antibody for the glucocorticoid receptor was omitted from the primary incubation medium.

The differential distribution of glucocorticoid receptors in the olfactory mucosa resembles the pattern of localization of mineralocorticoid (type I) receptors (Foster et al., *Assoc. Res. Otolaryngol. Abstr.* 17:33, 1994; Foster et al., *Soc. Neurosci. Abstr.*, 20: 1473, 1994; Kern et al., *Assoc. Res. Otolaryngol. Abstr.* 18:649, 1994) and Na, K-ATPase (Kern et al., *Brain Res.*, 546:8-17, 1991; Foster et al., *Assoc. Res. Otolaryngol. Abstr.* 17:33, 1995) observed in previous studies. The distinctive cell localization of the glucocorticoid receptor in the olfactory mucosa suggests a role of glucocorticoid hormones in regulating the function of these cells.

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686.4

MODULES FOR MOLECULES? TOPOGRAPHICAL ORGANIZATION OF THE ZEBRAFISH OLFACTORY SYSTEM.
F. Weth, H. Baier and S. Korsching*. Max Planck Institute for Developmental Biology, D-72076 Tübingen, Germany.

We are investigating the principles governing the primary representation of odor information in the olfactory epithelium and the olfactory bulb. To this end, we are analyzing the primary olfactory system of the zebrafish, *Danio rerio*, by a combination of axonal tracing and *in situ* hybridization.

We injected one identified glomerulus with DiI to trace back olfactory neurons connected to it. The labeled neurons are widely scattered over the sensory surface without apparent topographical order. However, the relative positions of the retrogradely labeled neurons are not completely random, as judged from measuring nearest-neighbor distances. Rather, spacing is observed: Olfactory neurons projecting to the same target site keep a minimum distance of roughly ten cell diameters from each other. As the spacing is unique to neurons terminating in the same glomerulus, it should be useful as an indicator of common projection.

Cloned odorant receptor molecules of the zebrafish belong to a gene family of less than 50 members and are homologous to their mammalian counterparts. As revealed by *in situ* hybridization, individual receptors are expressed by a small subset of sensory neurons, which are widely scattered over the olfactory epithelium. We are currently analyzing the nearest-neighbor distances of cells expressing monoallelic single copy receptor genes. If their distribution confirms the spacing rule obtained from retrograde tracing, this would indicate a common projection. In a more direct approach, we attempt to double-label olfactory neurons by retrograde labeling and *in situ* hybridization.

Ref: *J. Neurosci.* **14**, 219-230 (1994), *Proc. Natl. Acad. Sci.* **91**, 11646-50 (1994).

686.5

ACTIONS OF CARBON MONOXIDE AND CYCLIC GMP ON ODOR RESPONSES OF ISOLATED OLFACTORY RECEPTOR CELLS. F. Zufall*, G. M. Shepherd and T. Leinders-Zufall. Section of Neurobiology, Yale University School of Medicine, New Haven, CT.

There is increasing evidence supporting the function of carbon monoxide (CO) as a diffusible messenger in the vertebrate olfactory system. CO can act as a potent activator of olfactory cyclic nucleotide-gated (CNG) channels by stimulating cGMP formation in olfactory receptor neurons (ORNs) and this effect provides a sensitive mechanism for regulating excitable properties of ORNs (Leinders-Zufall et al., this meeting). Here we have examined the interaction of the CO-induced cGMP pathway with the odor-stimulated G-protein coupled cAMP pathway and tested whether this CO/cGMP system can influence the responsiveness of ORNs to odor stimulation. Odor responses from single salamander ORNs were obtained using the perforated patch technique. This permitted recording of both odor-induced membrane currents and membrane potentials so that sensory generator currents at the input level of the cell could be related to action potential discharges at the output level of the cells. ORNs that were voltage clamped to their resting potential responded with transient inward currents to short pulses of odor stimuli. In contrast micromolar doses of CO induced long-lasting tonic inward currents. Recovery from this CO effect was on the time scale of minutes. When odor-induced transient currents were tested during the long-lasting CO-induced currents, the odor responses were markedly diminished. This effect could be mimicked by low doses of 8-br-cGMP. Under current clamp conditions odor pulses induced depolarizing receptor potentials leading to transient action potential discharges. Micromolar doses of CO induced long-lasting depolarizations that modulated the spike discharge pattern induced by odor pulses resulting in most cases in a reduction of the firing rate. Our results indicate that the presence of CO leads to several discrete changes in the signaling properties of ORNs which, taken together, strongly influence odor responsiveness of the cells. Supported by grants from NIDCD (F.Z.) and from NIDCD and NIMH, NASA and NIDCD (Human Brain Project) (G.M.S.).

686.6

LOCALIZATION OF GLYCINE RECEPTORS AND GEPHYRIN IN THE RAT OLFACTORY BULB.
M. Sassoè-Pognetto*[1], M. Giustetto[1], J. Kirsch[2], and D. Cantino[1]. [1]Dept. of Human Anatomy and Physiology, Univ. of Turin, I-10126 Turin, Italy; [2]Max-Planck-Institute for Brain Research, D-60528 Frankfurt a.M., Germany.

The localization of glycine receptors (GlyRs) and the GlyR-associated protein gephyrin was investigated in the rat olfactory bulb (OB), using immunocytochemistry. The monoclonal antibody (mAb) 4a, which can recognize all of the known GlyR subunits (α1-3 , ß), produced a diffuse staining in mitral and tufted cells, as well as a punctate staining in the glomeruli. According to previous *in situ* hybridization data (Malosio *et al.*, EMBO J., 1991), we assume that this staining reflects the presence of the ß subunit of the GlyR. Surprisingly, electron microscopy showed that some of the presumed glutamatergic synapses made by olfactory nerve terminals onto the dendrites of mitral/tufted cells were immunopositive.

mAb 2b, which is specific for the strychnine-sensitive α1 subunit, produced no immunoreactivity in the OBs of adult and young (P3, P10) rats. Therefore, we conclude that the α1 subunit is not expressed in the OB.

mAb 7a, which is specific for gephyrin, produced a strong punctate labelling in the external plexiform layer and in the glomeruli. Immunoreactive puncta outlined the cell bodies and main dendrites of mitral and tufted cells. Electron microscopy showed that gephyrin is present in the synapses made by local interneurons (periglomerular and granule cells) with the dendrites of mitral/tufted cells. Since many periglomerular and granule cells use GABA as a neurotransmitter, it is likely that in the OB, as in the retina (Sassoè-Pognetto *et al.*, J. Comp. Neurol., 1995), gephyrin might be localized to GABAergic synapses.

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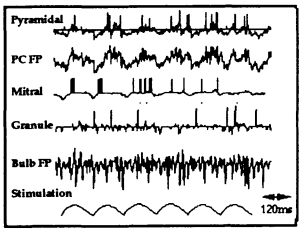
686.7

INTERACTIONS BETWEEN OLFACTORY BULB AND OLFACTORY CORTEX IN A NEURAL MODEL OF ODOR PROCESSING. Christiane Linster*(1), Michael E. Hasselmo(1) and Remi Gervais(2) (1) Dept. Psych., Harvard Univ., Cambridge MA 02138 (2) Lab. de Physiologie Neurosensorielle, CNRS, 69622 Villeurbanne.

Cholinergic modulation has been shown to have effects at various levels of the mammalian olfactory system. Behavioral studies have shown that cholinergic modulation is involved in olfactory short term memory in the olfactory bulb, as well as in olfactory learning in higher structures. Physiological experiments show the effect of cholinergic modulation on synaptic transmission in the olfactory bulb (OB) (Elaagouby et al., Neurosci., 56, 1991) and in the piriform cortex (PC) (Hasselmo , Brain. Res. 67, 1995). We have currently implemented biophysical simulations of the OB (Linster and Gervais, submitted) and the PC, both exhibiting realistic population dynamics (rapid field potential (FP) oscillations around 50-60 Hz in the OB as well as slow (3-8 Hz) and rapid (50-60 Hz) FP oscillations in the PC) and realistic single neuron responses (spontaneously and in response to olfactory input) (see Figure).

These models now allow us to investigate (i) the dynamic interactions between OB output neurons and the feedback of PC onto OB interneurons, (ii) synchronization of mitral cell spike trains synapsing on common pyramidal cells, (iii) effects of neuromodulator in both layers and (iv) synaptic changes related to odor processing.



686.8

FLAVOR AND THE FRONTAL CORTEX. R. Schul[1], Y. Dudai[1], and B.M. Slotnick*[2] [1]Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel, [2]Dept. of Psychology, The American University, Washington, D.C., USA.

Several lines of evidence indicate that orbitofrontal or insular cortex may be involved in the integration of odor and taste, the intraoral combination of which produces the perception of flavor. To assess this, rats were first trained using operant conditioning to respond at a high rate when they received a solution of NaCl and amyl acetate and to inhibit responding when only the odor or only the taste component was delivered. Concentrations of the stimuli were varied so that intensity could not be used as a discriminative cue. The maximum concentration of amyl acetate was below gustatory threshold as determined in tests with normal and olfactory bulbectomized rats. After training, experimental rats (n=4) received a unilateral olfactory bulbectomy, transection of the anterior limb of the anterior commissure and removal of the contralateral frontal cortex. Thus, these rats had no olfactory input to one hemisphere and no gustatory input to cortex in the contralateral hemisphere. Control rats received no surgery (n=5) or had all lesions in the same hemisphere (n=5). All controls showed good retention or rapid reacquisition of the flavor discrimination task and differences between operated and non-operated controls were not significant. In contrast, experimental rats had no retention of the task and showed no or only modest improvement in performance even after extensive retraining. In additional tests, all rats were able to discriminate the odor and taste component stimuli from water. These data suggest that there is a convergence of gustatory and olfactory input to frontal cortex which is essential for the integration of taste and smell. (Supported by a grant from the US-Israel Binational Science Foundation, Jerusalem to Y.D. and B.M.S.)

686.9

TASTE NEURONS IN INSULAR-OPERCULAR CORTEX OF THE ALERT CYNOMOLGUS MONKEY. Thomas R. Scott* and C.R. Plata-Salamán. Departments of Psychology and Biology and Program in Neuroscience, University of Delaware, Newark, DE 19716.

Primary gustatory cortex in the macaque is located in frontal operculum (FO) and adjoining anterior insula (AI). Here we report on the characteristics of taste-responsive neurons in this area. Taste cells constituted 5% of the neurons whose response properties were investigated. They occupied an area extending 4 mm posteriorly from the junction of orbitofrontal cortex with the FO, 3 mm mediolaterally, and 7 mm in the dorsoventral plane. Most action potentials were only 200-500 μV. The mean spontaneous rate was 3.9 ± 4.9 spikes/sec. There was no indication of chemotopic organization in the gustatory cortex. Of 364 taste-responsive neurons, glucose was the most effective of the basic stimuli for 39% (n=142), NaCl for 29% (n=107), quinine for 20% (n=74) and HCl for 13% (n=49). Neurons most responsive to glucose and NaCl were more narrowly tuned ($p < 0.01$) than those sensitive to quinine and HCl. Across all stimuli, the ratio of excitatory to inhibitory responses was about 10:1. The mean response across all cells rose monotonically with increasing stimulus intensity, but the slopes of intensity-response functions were lower than those from human psychophysical studies. When slopes were calculated exclusively from the responses of cells in the appropriate subgroup, however, they matched intensity-response functions from humans. Thus, perception of the four basic taste stimuli is probably carried by the subgroup of neurons most sensitive to a particular quality, rather than by the responses of all cells. An analysis of patterns of activity evoked by a wide range of stimuli indicated that taste quality coding in the macaque is quite similar to that in human.

Supported by a research grant from the NSF.

686.10

TESTOSTERONE DIFFERENTIALLY REGULATES PHEROMONE INDUCED FOS EXPRESSION IN LIMBIC REGIONS OF MALE AND FEMALE SYRIAN HAMSTERS. J.M. Fiber* and J.M. Swann. Institute of Animal Behavior and Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102.

Exposure to female hamster vaginal secretions (FHVS) specifically induces Fos expression in the posteromedial subdivision of the bed nucleus of the stria terminalis (BNSTpm), the posterior subdivision of the medial nucleus of the amygdala (MeP), and the magnocellular subdivision of the medial preoptic nucleus (MPNmag) of male hamsters. We have also previously reported a sex difference Fos expression in these regions after FHVS exposure. In the present study we sought to determine the role of testosterone in regulating pheromone induced Fos expression in both males and females.

Adult male and female hamsters were gonadectomized and treated with a 20mm testosterone capsule (TC) or with an empty capsule (EC). Twelve weeks later, animals were exposed to FHVS or given no stimulus (controls). Brain tissue was processed for Fos immunocytochemistry.

Our results indicate a sex difference in testosterone regulation of pheromone stimulated Fos expression in the BNSTpm, MeP, and MPNmag. Males +TC (n = 5) show a greater number of Fos immunoreactive cells (IR) within the MPNmag than females +TC (n = 5) or females + EC (n= 4); males + EC (n = 5); and all control groups (n = 3 for each group) ($p > .01$). Both the BNSTpm and MeP show FHVS induced Fos expression in males + TC and + EC above that of controls and FHVS exposed females + EC ($p < .01$). In FHVS exposed females + EC, the BNSTpm and the MeP are not stimulated to express Fos above controls, however testosterone acts in females to reverse this effect ($p < .01$). Thus, circulating testosterone influences pheromone stimulated Fos in the BNSTpm and MeP of females, and in the MPNmag of males. These differences in pheromone stimulated Fos IR implicate these brain regions in subserving testosterone regulated behavioral sex differences in response to exposure to FHVS.

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FORMATION AND SPECIFICITY OF SYNAPSES I

687.1

MORPHOGENESIS OF AN IDENTIFIED MOTONEURON IN RESPONSE TO TARGET MANIPULATION. J.J. Fernandes and H. Keshishian*. Biology Dept., Yale University, New Haven, CT 06520.

We are examining nerve-muscle interactions during the development of the six adult Dorsal Longitudinal flight Muscles (DLMs) of *Drosophila*. The fibers develop during metamorphosis from three persistent larval muscles, and are innervated by 5 motoneurons. The two most dorsal fibers (DLMs e and f) are innervated by a single motoneuron, MN5. Using intracellular Lucifer yellow dye fills, we examined the target-dependence of MN5's development during metamorphosis (from 18-36h APF; after puparium formation). The peripheral synaptic arbor is confined to muscle fibers e and f throughout the stages examined, and corresponds to the entire component previously characterized by anti-HRP. In the CNS, the dendritic arbor arises about 10-12 hrs after the differentiation of the peripheral motor endings, indicating that they do not develop in synchrony. Dendrites appear at 26h APF, at a time when the peripheral arborization on the muscle fibers is well elaborated. We next examined the effects of muscle fiber deprivation/alteration on MN5. Following laser ablation of the larval template for DLMs e and f, adult fibers develop de novo, although in a delayed fashion. Innervation to these fibers is corresponding delayed (Fernandes et al, Soc. Neurosci. Abst. '94). In the CNS we did not observe any effect on the dendritic arbors during stages 26-30h APF. We are further examining CNS arbor development at earlier stages to determine whether this neuronal compartment is altered in response to peripheral delays. Target deprivations lead to ectopic motor endings made onto the remaining fibers, as observed using anti-HRP. Our dye fills show that MN5 can be the source of these ectopic synapses. These results show that timing of MN5 motor ending development is closely tied to the developmental state of the innervated muscle fibers, and that this compartment of the neuron develops under local cues.

687.2

The *Drosophila* mutation *Passover* alters gap junction function in the Giant Fiber pathway Yi-an Sun* and R. J. Wyman. Department of Biology, Yale University, New Haven, CT 06511

Mutations of the *Drosophila* gene *Passover* and of a *C. elegans* homolog, *unc-7*, alter specific electrical synapses in their respective organisms. The proteins made by these genes (Cell 73:967-977; Genetics 133:527-541), and several other sequence similar genes, may have a structure similar to the gap junction structural molecule, connexin (Trends in Genetics 10:303).

In *Drosophila*, an electrical synapse connects the Giant Fiber (GF) and the tergotrochanteral motoneuron (TTMn) (J. Neurophys. 44:405; J. Neurocytol. 9:753). In flies mutant or the *Passover* gene, the synapse is abnormal. In this report, we show that the Giant Fiber also makes electrical synapses with a commissural tract in the brain. In *Pas* flies, the synapses connecting the Giant Fiber to the TTMn and the commissure are either absent or malfunctional.

We injected cobalt and HRP into the TTM muscle and into the mesothorax. The giant fibers ascend to the brain from the cervical connective and then fan out in a dorsolateral trajectory. In wild type, but not in *Pas*, cobalt passes from the fingers of the GF's fan through gap junctions into several fibers of a commissure just dorsal to the esophagus. The commissure fibers connect the two GFs to each other. HRP, which does not pass gap junctions, does not pass into the commissure fibers. Cobalt also passes from the TTMn to the GF in wild-type, but not in *Pas*. We conclude that the gap junctions in these two electric synapse are malfunctional in *Pas*. Supported by NIH NS-07314; NSF IBN-9213387.

687.3

ROLE OF MOTOR NEURON GAP JUNCTIONAL COUPLING IN NEUROMUSCULAR SYNAPSE ELIMINATION. R.J. Balice-Gordon*, J. Pines and S. Blumenthal. Dept. Biology, Univ. of Penn., Phila., PA 19104-6018.

During development and adult reinnervation, transient multiple innervation of neuromuscular junctions is followed by a period of synapse elimination which continues until single innervation is established. We are interested in understanding the modulation of this process by activity. Synchronous activity among the different inputs to a neuromuscular junction, perhaps mediated by transient electrical coupling among motor neurons, may be important for the establishment and transient maintenance of multiple innervation, while asynchronous activity among synapses may lead to synapse elimination. To begin to evaluate this hypothesis, we used immunohistochemistry to show that before and at birth, ca. 80% of the motor neurons in the mouse lumbar spinal cord express the gap junction protein cx32 (antibody provided by Dr. D. Paul.). Intracellular recording has shown that motor neurons are electrotonically coupled during this time (c.f. Fulton & Walton, 1986). The percentage of motor neurons expressing cx32 declined after birth until by P14, as in adults, few if any positive motor neurons were detected. Similar results were obtained from the sternomastoid motor pool. Given that multiple innervation is transiently recapitulated during adult reinnervation, we used cx32 immunohistochemistry to determine whether gap junction proteins are re-expressed in adult motor neurons following double nerve crush. One week after crush, when multiple innervation is widespread, ca. 70% of motor neurons ipsilateral to the crush stained positively. Two weeks after crush, when synapse elimination is underway, 50% of motor neurons were positive; four weeks after crush, when single innervation is re-established, less than 10% of motor neurons were positive. In both development and adult reinnervation, cx32 expression in motor neurons was the most widespread during the time multiple innervation is present, and both disappeared with a similar time course. We are using physiological methods to characterize the extent of electrotonic coupling and the synchrony of activity among motor neurons during development and reinnervation.

687.4

***IN VIVO* EXAMINATION OF ACETYLCHOLINE RECEPTOR TURNOVER IN DENERVATED NEUROMUSCULAR JUNCTIONS USING CALIBRATED FLUORESCENCE IMAGING.** S.M.Culican*, S.G.Turney, and J.W.Lichtman. Department of Anatomy and Neurobiology, Washington University School of Medicine, St.Louis, MO 63110.

Using a quantitative imaging technique to follow changes in the absolute intensity of a fluorescence signal, we have studied the turnover of acetylcholine receptors (AChRs) labelled with rhodamine conjugated alpha-bungarotoxin (R-BTX, an irreversible ligand) in neuromuscular junctions of living mice. This technique allowed measurement of the intensity of fluorescence in a defined junctional region, permitting assessment of changes in AChR density that were independent of changes in junctional size. With this method we have found that at sites within innervated junctions the half-life of AChRs is approximately 8 days, whereas at sites within denervated junctions the half-life of AChRs is approximately 2-3 days.

AChRs at denervated junctions are a mixed population: some receptors are the remanent of those present in the membrane before the denervation, while others have been inserted after denervation. Several studies have suggested that these two populations respond differently to denervation. To follow AChRs that were in the membrane before denervation, we labelled them with R-BTX just before nerve cut. Conversely, to examine new receptors, we pre-labelled denervated junctions with unlabelled BTX three days after denervation, blocking all the receptors that were present in the membrane prior to denervation. The newly inserted AChRs were labelled with R-BTX 7 days after denervation. For both populations, intensity measurements at individual junctional sites were begun ten days after denervation. At subsequent timepoints the junctional sites were relocated and re-imaged. Changes in the absolute intensity of the rhodamine label between views was determined at each site. Our results suggest that both populations of AChRs are lost from the membrane at the same rate ($t_{1/2}$ approx. = 2-3 days).

687.5

NEURONAL AGRIN INDUCES LATE AS WELL AS EARLY EVENTS IN POSTSYNAPTIC APPARATUS FORMATION IN DENERVATED MAMMALIAN SKELETAL MUSCLES. I. Cohen, M. Rimer, T. Lomo, C.R. Slater and U.J. McMahan*. Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Evidence derived mainly from in vitro experiments has led to the hypothesis that agrin released from motor axon terminals directs the initial events of postsynaptic apparatus formation at developing neuromuscular junctions by inducing myotubes to make local aggregates of AChRs and AChE and certain other proteins. Determining by in vitro studies whether agrin induces later events such as the formation of junctional folds and the accumulation of myonuclei is made difficult by the low survival rate and uncertain behavior of myotubes maintained for prolonged periods in culture medium. An in vivo preparation that may permit a detailed analysis of neuronal agrin's role in all aspects of postsynaptic apparatus formation is the denervated adult rat soleus muscle, in which myofibers are competent to form postsynaptic apparatus throughout much of their length. We injected cDNA coding for neuronal agrin into the extrajunctional re-gions of such muscles. The cDNA included a region encoding signal sequence to direct the protein into the myofibers' secretory pathway. By 7d, muscle fibers expressing n-agrin and those adjacent to them had on their surface colocalized aggregates of AChR, AChE and several other postsynaptic proteins as detected by immunofluorescence microscopy. By 6wks the AChR/AChE aggregates induced by n-agrin were accompanied by a preferential localization of myonuclei and the presence of infoldings of the plasma membrane similar in size and shape to junctional folds, as determined by electron microscopy. These results make it likely that the interaction of axon-released n-agrin with agrin receptors on myotubes at the developing neuromuscular junction induces at least some late as well as early events in postsynaptic apparatus formation. Further experiments with transfected soleus muscles may provide insights as to whether n-agrin plays a role in the induction of other late events such as changes in the gene regulation of AChR subunits.

687.6

CLUSTER-INDUCING ACTIVITY OF LAMININ VERSUS AGRIN. M.W. Cohen*. Dept. of Physiology, McGill Univ., Montreal, Quebec. H3G 1Y6.

Since laminin and agrin compete for binding to muscle α-dystroglycan (Cell 77:675-686, 1994) the question arises whether laminin affects the distribution of surface molecules in the same way as agrin. When embryonic Xenopus myocytes were cultured without agrin or laminin, clusters of acetylcholine receptors (AChRs) had colocalized laminin-binding sites (LBS) and phosphotyrosine (PY). The myocytes also exhibited a widespread distribution of microclusters of LBS which did not contain any detectable AChR or PY stain. When agrin (a gift from E.W. Godfrey and R.M. Nitkin) was included in the culture medium there was a large increase in the incidence of clusters containing AChRs, LBS and PY, and not much decline in the density of LBS microclusters. By contrast, laminin caused a large decline in the density of the widespread LBS microclusters and a concommitant increase in the incidence of larger clusters of LBS. AChRs and PY were rarely detected at these laminin-induced clusters of LBS. The results suggest that (1) laminin has cluster-inducing activity which is different from that of agrin, (2) the clustering of LBS, unlike the clustering of AChRs, does not require tyrosine phosphorylated proteins, and (3) unclustered LBS on the cell surface are mobile, as previously suggested for unclustered α-dystroglycan (J. Cell Biol. 129: 1093-1101, 1995). (Supported by MRC of Canada)

687.7

CRANIN, A LAMININ BINDING MEMBRANE PROTEIN: IDENTITY WITH DYSTROGLYCAN AND REASSESSMENT OF ITS CARBOHYDRATE MOIETIES. N. R. Smalheiser* and E. Kim. Dept. of Pediatrics, University of Chicago, Chicago, IL 60637.

Cranin was described in 1987 as a membrane glycoprotein expressed in brain and many other tissues, which binds laminin with high affinity in a calcium-dependent manner. Dystrophin-associated glycoprotein ('dystroglycan') is a laminin-binding protein cloned in 1992 whose relation to cranin has remained uncertain. Using cranin purified to homogeneity from sheep brain, we now show that cranin is a form of dystroglycan, based on amino acid sequencing of 120 kDa and 43 kDa bands, and on immunological cross-reactivity with antibodies raised against synthetic peptides derived from dystroglycan sequences. These data also localize the N-terminus of ß-dystroglycan to amino acid residue 654. We find that brain alpha-dystroglycan is tightly associated with membranes, and localizes to regions of CNS synaptic contact as assessed by immunocytochemistry of rat cerebellum. Brain alpha-dystroglycan expresses high mannose/hybrid N-linked saccharides, terminal GalNAc residues, and the HNK-1 epitope. Though dystroglycan has been presumed to be a proteoglycan, the amino acid sequence, pI, O-sialoglycoprotease susceptibility, lectin-binding and laminin-binding properties of brain dystroglycan are more typical of mucin-like proteins. Furthermore, using CHO cell lines deficient in xylosyltransferase and galactosyltransferase I, which are required for glycosaminoglycan biosynthesis, it is shown that chondroitin sulfate and heparan sulfate are not critical for laminin binding, and indeed are apparently not expressed in dystroglycan from CHO cells. Supported by NIH HD 09402, NS 26055 and the Brain Research Foundation, Inc.

687.8

IDENTIFICATION AND LOCALIZATION OF ARIA RECEPTORS IN SKELETAL MUSCLE AND MUSCLE CELL LINES G. Corfas*, T.S. Khurana, C. Lai* and G.D. Fischbach. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115 and *The Scripps Institute, La Jolla, CA 92037.

ARIA is a protein that promotes the synthesis of acetylcholine receptors in muscle, and thus may play a role in the formation and maturation of the neuromuscular junction. ARIA is a member of a family of factors that are ligands for members of the EGF receptor (EGFR) family, i.e. HER2, HER3 and HER4. In order to determine which of these receptors are involved in ARIA's action on skeletal muscle we studied the expression and localization of these proteins on skeletal muscle, and their ARIA-induced tyrosine phosphorylation on L6 cells.

We have found that in lysates of ARIA-treated L6 cells, antibodies directed against all these receptors are capable of precipitating a portion of the p185 signal. An antibody raised against a peptide shared by all the members of the EGFR family precipitated all the p185 signal, which appears as a doublet. Anti-HER2 antibodies precipitated mostly the lower band of p185, while anti-HER3 antibodies precipitated mostly the higher band. Anti-HER4 antibodies precipitated a much smaller portion of the tyrosine phosphorylated protein than antibodies directed against the other receptors. In all cases, the only tyrosine phosphorylated protein recognized by these antibodies was p185. We have studied the expression of these receptors in skeletal muscle using immunocytochemistry with anti-receptors antibodies. HER2 and HER4 immunoreactivity appears to be concentrated at neuromuscular junctions. HER3 antibodies gave inconclusive results.

These results suggest that HER2, HER3 and HER4 may participate in the signalling of ARIA on muscle cells, and that at least some of these receptors may be concentrated at mature neuromuscular junctions.

687.9

NEUROTROPHIC REGULATION OF ARIA EXPRESSION.

J. A. Loeb and G. D. Fischbach.* Harvard Medical School, Dept. of Neurobiology, Boston, MA 02115

ARIA, or acetylcholine receptor-inducing activity, initiates a cascade of events leading to the expression of postsynaptic components of the neuromuscular synapse. These include increasing the expression of AChRs and sodium channels. We now have evidence suggesting that neurotrophins from the postsynaptic muscle cell augment the expression of ARIA in the presynaptic neuron. Using embryonic rat ventral horn cultures containing motor neurons expressing ARIA, we have found that the muscle-derived neurotrophins NT-3 and BDNF increase the amount of ARIA mRNA by up to 3-fold. This effect peaks at 4 hours of treatment at a half maximal concentration of 10-100 pM. Under identical conditions there is no change in messages for the low affinity receptor for NGF, or other presynaptic proteins. Furthermore, NGF, LIF, CNTF, or ARIA do not alter ARIA mRNA expression in these cultures. BDNF and NT-3 also increase the amount of ARIA protein activity secreted into the media of these cultures. We have found no evidence for a reciprocal effect in that ARIA does not affect BDNF or NT-3 expression in embryonic cultured rat myotubes. Even though these cultures respond to ARIA by inducing the phosphorylation of an 185 KDa phosphoprotein, the levels of BDNF and NT-3 mRNA are constant after treatment for up to 48 hours with ARIA. Such interactions between the neurotrophins and ARIA could serve to reinforce specific developmental programs at synaptic contact sites.

687.10

EXCITATORY SYNAPSE FORMATION IN CULTURED SPINAL NEURONS. R.J. OBrien*, and R.L. Huganir. Dept. of Neuroscience and Neurology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

While much is known about the mechanism of synapse formation at the neuromuscular junction, little is known about the mechanisms involved in the formation of excitatory central synapses. We have elected to use cultured rat spinal cord neurons to investigate how AMPA type receptor subunits are regulated during synapse formation. Neurons from E17 rat spinal cords grown at low density on glial feeder layers, form excitatory synapses within 4 days. Using subunit specific antisera, these synapses are seen to be composed of the AMPA receptor subunits GluR1, GluR2/3, and GluR4 contained within a cluster on the postsynaptic dendrite. Receptor clusters develop only when juxtaposed to a presynaptic release site (determined by synaptophysin staining) and do not develop spontaneously. The concordance between excitatory presynaptic release sites and postsynaptic clusters is nearly 100% both in heterogeneous cultures and in neurons grown in isolated islands. Inhibitory interneurons cause no clustering of AMPA type receptors. Activation of NMDA or AMPA type receptors does not appear to be required for this clustering, and clustering cannot be induced by isoforms of AGRIN active at the neuromuscular junction. We are presently involved in correlating electrophysiologically defined "hot spots" with these postsynaptic clusters.

687.11

SYNAPTOGENESIS BY CELLS DERIVED FROM THE HUMAN NT2 CELL LINE. Cha-Min Tang*[1], Michael Margulis[1], Rebecca Hartley[2], and Virginia M.-Y. Lee[2]. Dept. of Neurology, U. of Maryland[1] and Dept. of Pathology, U. of Pennsylvania[2].

NT2 cells are believed to be immortalized equivalent of neural progenitor cells in the developing human nervous system. Treatment with retinoic acid can induce NT2 cells to commit to terminal differentiation into neurons (NT2N). While NT2N cells under current in vitro culture conditions can express a sequence of neuronal markers that recapitulates the maturational steps in the developing nervous system, they do not express some late events in neuronal maturation (i.e. functional synapses). We examined the maturation of NT2N cells under different culture conditions. When NT2N cells were co-cultured with astrocytes patch clamp recordings from them revealed synaptic activity that were not previously seen. These synaptic currents exhibited the biophysical and pharmacological characteristics of glutamergic synaptic transmission. Synaptic currents suggestive of $GABA_A$-mediated synaptic signals were also observed. These electrophysiological data were supported by positive immunohistochemical staining for synaptophysin, a synaptic vesicle protein. When NT2N cells were co-cultured with striate muscle they formed functional synapses that are reversibly blocked by curare. These findings suggest that NT2N cells have the capacity to express functional synapses and a variety of neurotransmitters.

687.12

PRESYNAPTIC INFLUENCE ON POSTSYNAPTIC CHANNEL KINETICS IN CULTURED SYMPATHETIC NEURONS.

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The nicotinic channels of B- and C-cells, the principal neurons in bullfrog lumbar sympathetic ganglia, have different kinetics (J.Neurosci. 6:590,1986). B- and C-cells are innervated selectively by B- and C-preganglionic neurons, which are located in separate regions of the adult spinal cord (J.Comp.Neurol. 268:71,1988). This study examined the influence of presynaptic inputs on postsynaptic channels by comparing synaptic currents of B- and C-cells that had been co-cultured with spinal cord segments that contained either B- or C-preganglionic neurons.

Miniature excitatory post synaptic currents (mEPSCs), evoked by focally applied sucrose, were recorded from ganglion cells using the tight seal whole cell voltage clamp technique. The mean (±SEM) decay rates of mEPSCs recorded from ganglion cells co-cultured with B- or C-preganglionic neurons were 6.08 ± 0.25 (n=42) and 8.26 ± 0.43 (n=27) ms, respectively ($p<0.0001$). These results suggest that the kinetics of synaptic channels expressed in cultured sympathetic neurons are influenced by the type of innervating preganglionic axon. Future work will examine the influence of presynaptic axons on channels expressed by ganglion cells from early stage tadpoles.

687.13

NEUROSERPIN, AN AXONALLY SECRETED SERINE PROTEASE INHIBITOR

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Axonally secreted macromolecules have been implicated in neural development as mediators of axon outgrowth, remodelling of the extracellular matrix, and formation, elimination or competition of synapses. Here we report on the purification and the molecular cloning of neuroserpin, a neuronal serine protease inhibitor. Neuroserpin (previously denoted Axonin-2) had been identified in a compartmented cell culture system as an axonally secreted glycoprotein with an apparent molecular weight of 54-60 kD (Stoeckli et al., Eur. J. Biochem. 180: 249-258 (1989)). Based on coordinates and spot morphology in two-dimensional gel electrophoresis, neuroserpin was purified from chicken embryo vitreous fluid in a three step chromatographic procedure. The amino acid sequences of the amino terminus and three internal peptides were determined and used to design degenerated primers with which a 1000 kb fragment of the neuroserpin cDNA was amplified from chicken retina mRNA by means of RT-PCR. With the PCR-product as a probe, a full length cDNA of neuroserpin was isolated from a chicken brain cDNA library. The nucleotide and the deduced amino acid sequence qualified neuroserpin as a member of the serpin family of the serine protease inhibitors. The closest relatives are glia-derived nexin/protease nexin-1 and PAI-1 with an amino acid sequence identity of 35% and 34%, respectively. Primary structural features suggest that neuroserpin is heparin-independent and targeted against trypsin-like proteases. *In situ* hybridization revealed neuron-specific expression of neuroserpin during the late stages of neurogenesis. Together, the results implicate neuroserpin as a regulator of proteolytic events subserving processes of neural development such as synapse elimination, remodelling, or competition.

688.1

PROSTAGLANDIN INACTIVATION IN THE PERINATAL BRAIN: ROLE OF THE CHOROID PLEXUS. N. Krunic[1,2], I. Bishai[1], S.L. Adamson[2] and F. Coceani[1]*. [1]Div. of Neurosciences, Hosp. for Sick Children, Toronto, On., Canada M5G 1X8. [2]Samuel Lunefeld Res. Inst., Mount Sinai Hospital, Toronto, On., Canada M5G 1X5.

In early gestation, prostaglandins (PGs) are most likely inactivated by local catabolic enzymes. *This enzyme activity disappears by birth and, in* adults, PGs are actively transported across the blood-brain barrier and choroid plexus for catabolism in the periphery. We investigated whether PG transport occurs prenatally and if it increases at birth in association with the marked decrease in central PGE_2 levels. PG uptake in the choroid plexus from fetal (~136 d; term 145 d), newborn (3 d), and adult sheep was studied in vitro using [^{3}H]$PGF_{2\alpha}$ as substrate and [^{14}C]sucrose as extracellular marker. Fetal choroid plexus accumulated $PGF_{2\alpha}$, reaching steady-state at 30-60 min with a tissue-to-medium ratio (T/M) of 5.3 ± 0.5 (n=8). Newborn results were similar (T/M=7.0 ± 1.4, n=7). Uptake in the adult reached steady-state at 60-90 min (T/M=13.6 ± 1.3, n=8) and was significantly higher than fetus and newborn ($p<0.05$). Extracellular volume was constant at all ages (T/M=0.44 ± 0.02, n=23). Catabolism of $PGF_{2\alpha}$ to its inactive 13,14-dihydro-15-keto (15KD) metabolite was analyzed by thin-layer radiochromatography. Catabolism (radioactivity of 15KD expressed as %total) decreased significantly with age from fetus (59 ± 4%, n=8), to newborn (33±2%, n=7) to adult (no catabolism). $PGF_{2\alpha}$ uptake by the fetal choroid plexus had a high capacity (saturation at 40-60 µM) and was significantly inhibited by probenecid (1 mM, $p<0.05$). We conclude that the PG transport system of the choroid plexus develops in utero in sheep. PG uptake in vitro is lower, whereas catabolism is higher, in the perinatal period than in the adult. There is no abrupt increase in PG uptake at birth, which implies this mechanism is not responsible for the rapid decrease in central PGE_2 at this time. (NK supported by SIDS and Genesis).

688.2

EXPRESSION OF BRAIN CAPILLARY GSH TRANSPORT IN XENOPUS LAEVIS OOCYTES. R.Kannan*, J-R.Yi, Y.Li, D.Tang, B.V.Zlokovic and N.Kaplowitz. Depts. of Med., and Neurol. Surg., USC Sch. of Med., and VA Outpatient Clinic, Los Angeles, CA 90033.

We had previously shown carrier-mediated glutathione (GSH) uptake *across the blood-brain barrier (BBB) in two animal models using the rapid bolus injection technique (rat) and in situ vascular brain perfusion (guinea-pig). The* present study deals with expression of brain capillary GSH transport in Xenopus laevis oocytes. Oocytes were injected with either mRNA (30 ng) from bovine brain capillaries or water and maintained at 18°C for 3 days. Expression of GSH uptake in NaCl medium was observed as compared to water controls in 1 h incubations. The molecular form of uptake was predominantly as intact GSH in gamma glutamyltranspeptidase inhibited (acivicin) and non-inhibited conditions. GSH transport was partially (~40%) inhibited by removal of sodium (sucrose medium). In separate studies with bovine brain capillary mRNA-injected oocytes preloaded with GSH, efflux of GSH at 31.9%/h (water <2%/h) corresponding to a rate of 5.3 nmol/oocyte/h, was observed. The presence of a recently cloned rat canalicular GSH transporter (RcGshT) in rat whole brain, and guinea-pig and bovine brain capillaries was demonstrated by Northern blot analysis. Size fractionation of bovine brain capillary poly (A)$^+$RNA (as cDNA) yielded three distinct fractions showing GSH transport activity, one of which was RcGshT, while the other two were hitherto unidentified GSH transporters. The active size fractions showed carrier-mediated GSH transport; data on their kinetic parameters, ion requirements and inhibitor specificities will be presented. These studies provide a basis for a better understanding of brain GSH homeostasis with respect to the nature of GSH transporters at the BBB and may have impact in development of therapeutic strategies for GSH delivery to the brain.

Supported by VA Medical Research Funds and the Hoover Foundation.

688.3

HIGH GLUT1 GLUCOSE TRANSPORTER EXPRESSION IN HEMANGIOBLASTOMA ENDOTHELIA. E. M. Cornford*, S. Hyman, K. L. Black, M. E. Cornford, H. V. Vinters and W. M. Pardridge. UCLA School of Medicine, Los Angeles, CA 90095; West Los Angeles V. A. Medical Center, Los Angeles, CA 90073; and Harbor-UCLA Medical Center, Los Angeles, CA 90509.

Light microscopic immunochemistry indicated the enriched presence of the GLUT1 glucose transporter isoform throughout the central endothelia in a resected hemangioblastoma. Glial fibrillary acidic protein (GFAP) was observed only at the tumor border; no GFAP-reactivity was seen in stromal cells, pericytes or endothelia in central tumor regions. Quantitative EM immunogold analyses of GLUT1-positive sites per *u*meter of capillary membrane confirmed the GLUT1 transporter was highly enriched in tumor endothelial cell membranes. Immunogold EM using albumin (MW 65000) indicated that HSA moved freely from the vascular lumen into pericapillary regions, confirming the "leaky" barrier (seen in clinical neuro-imaging). GLUT1 is generally said to be more concentrated in erythrocytes than in any other cell, but GLUT1 in these tumor capillaries was more than 2-3-fold higher than seen in human red cells. We conclude that in the absence of GFAP, a high Glut1 density characterizes this tumor. The increased immunoreactive GLUT1 seen suggests this may be a useful model system for studying BBB GLUT1 regulation; some tumor-derived factor may induce GLUT1 expression in the hemangioblastoma endothelium.
Supported by NIH grant NS 25554

688.4

TRANSLATIONAL ACTIVATORS IN 5'- AND 3'-UNTRANSLATED REGIONS OF GLUT1 GLUCOSE TRANSPORTER mRNA. R.J. Boado* and W.M. Pardridge. Dept. of Medicine and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Recent studies have indicated that the blood-brain barrier GLUT1 glucose transporter is under post-transcriptional regulation. To undertake the functional mapping of the GLUT1 transcript, the present investigation studied the translational efficiency of full-length synthetic human (h) GLUT1 mRNA, and both 5'- and 3'-untranslated region (UTR) deleted mRNAs. Translation of 10 ng full-length hGLUT1 capped-mRNA in rabbit reticulocyte lysate produced 0.161 ± 0.013% TCA precipitable material (mean ± S.E., n = 3) after 30 minutes incubation at 30°C. Deletion of 5'- and 5'-/3'-UTR completely blocked the translation of these GLUT1 transcripts. The putative role of these hGLUT1 5'-UTR cis-acting elements was studied using the luciferase expression vector pGL2. DNA corresponding to the hGLUT1 5'-UTR generated by PCR was subcloned at the HindIII site of pGL2 located upstream of the luciferase 5'-UTR. Transfection of brain endothelial cultured cells with pGL2 containing the complete [nucleotides (nt) 1-171] hGLUT1 5'-UTR markedly increased the expression of luciferase (60.7 ± 14.3 vs. 10.7 ± 0.7 pg luciferase/mg protein, respectively). Insertion of hGLUT1 5'-UTR in reverse orientation induced no changes in the expression of luciferase (9.4 ± 1.7 pg/mgp), and the insertion of an unrelated 171 nt decreased its expression. Insertion of nt 96-171 of the hGLUT1 5'-UTR retained most of the stimulatory effect (41.3 ± 10.0 pg/mgp). In parallel experiments, nt 2100-2300 of the bovine (b) GLUT1 3'-UTR were inserted at the PflMI site of pGL2, which is located downstream of the luciferase 3'-UTR. This region of the bGLUT1 3'-UTR encompasses the binding domain of a 88 kDa C6 cytoplasmic trans-acting factor. Transfecton of C6 cells with this construct markedly increased (> 300%) the expression of luciferase. Conclusion: The present data provide evidence suggesting that the 5'- and 3'-UTR of the GLUT1 mRNA contains cis-acting elements involved in a translational activation of the GLUT1 gene in mammalian cells.

688.5

A NEW VASOGENIC BRAIN EDEMA (VBE) MODEL AND RELATION TO ENZYMATIC BARRIER. B.X.Lu* and X.D.Tang. Dept. of Neurol., Nanfang Hospital, First Military Med. Univ., Guangzhou 510515, PR China.

At present, there is still no very satisfactory VBE model. Here reported was a new VCE model recently developed in this laboratory. Phenylephrine (1.2mg/kg, i.p.) was injected into spontaneous hypertensive stroke prone rats (SHRsp). Results demonstrate that the permeability of blood-brain barrier (BBB) to macromolecules, assessed by Evans blue, Indian ink and ^{125}I bovine serum albumin, was progressively increased and reached a maximum at 0.5-1 hrs after drug injection. The density of cerebral capillary of drug-treated rats, analysed by computer (Quantimet 500+), was about 2 times larger than that of untreated rats. Water content of gray and white matter, determined by a Moisture Analyzer (Denver Ins.), of control group versus drug-treated group was 59.04±9.51%, 60.97±8.67% (n=27) and 69.59±12.05%, 74.27±7.99 % (n=25), respectively. Student's *t*-test revealed a marked statistical significance between the two pairs of values (P=0.014, 0.0003). We further conducted another experiment using SHRsp injected with neostigmine methylsulfate (0.8 mg/kg, i.p.) to test the relation between enzymatic barrier damage (we have found that 12 enzymes of cerebral capillaries changed on VBE, not reported here) and VBE. Just as we expected, neostigmine could alleviate brain edema, which is suggested to result from the inhibition of BBB cholinesterase. Our results suggest that the phenylephrine-induced cerebral edema is a suitable VBE model and insult of enzymatic barrier could influence the formation of VBE.

688.6

ADHESION OF AFRICAN TRYPANOSOMES TO HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS. B. Zünkeler, Z. Fabry, K.A. Follett*, J. Brayton, J.C. VanGilder, J.E. Donelson, M.N. Hart. Laboratories of Neuropathology, Neurosurgery, and Biochemistry, University of Iowa, Iowa City, IA 52244

African sleeping sickness is thought to be due to a direct effect of *Trypanosoma brucei gambiense* and *rhodesiense* on anatomical structures in the vicinity of the third ventricle and, in later stages, on the entire brain. To cause disease, the parasite must first attach to and migrate through the cerebrovascular endothelial layer. In this study we address the mechanism of trypanosome adhesion to human brain microvascular endothelial cells by characterizing adhesion molecules that participate in this interaction. Parasites were passaged in rats and stained with ethidium bromide prior to incubation with subconfluent and confluent monolayers of human brain microvascular endothelial cells, (passage 5-10, *Ulex europaeus* agglutinin-phycoerythrin FACS isolated). Attachment of parasites was quantified by fluorescence microscopy. Adhesion of parasites could be blocked by 83% at 4 hours by monoclonal antibody to E-selectin, CD 62-E (ELAM1) and by 48% by monoclonal antibodies to vascular cell adhesion molecule, CD 106 (VCAM1). Anti CD 44 and anti CD 62-P antibodies had no effect on the adhesion of parasites to endothelium. These findings suggest that attachment of trypanosomes to human brain endothelium is mediated at least in part by cellular adhesion molecules, including ELAM1 and VCAM1.

688.7

CATIONIZED HUMAN IMMUNOGLOBULINS AS A TREATMENT FOR CEREBRAL ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS): EVALUATION OF PHARMACOKINETICS, EFFICACY, AND TOXICITY IN SCID-hu MICE. W.M. Pardridge*, J. Yang, J. Buciak, and Y.-S. Kang. Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

The treatment of cerebral AIDS requires that drugs undergo transport through the brain capillary endothelial wall, which makes up the blood-brain barrier (BBB) in vivo. Antibodies are potential AIDS therapeutics and cationized antibodies undergo transport through the BBB via absorptive-mediated transcytosis (Proc. Natl. Acad. Sci. 86:4761, 1989). Cationized human immunoglobulins have inhibitory effects on the replication of the human immunodeficiency virus (HIV) in human lymphocytes in tissue culture (J. Infect. Dis. 170:563, 1994). The in vivo efficacy of cationized human immunoglobulins in the SCID-hu mouse model was evaluated. Immunoglobulins from non-infected humans and from HIV-infected individuals were cationized. A pharmacokinetic analysis showed that cationized immunoglobulins have a markedly reduced mean residence time in plasma and a marked increase in organ uptake compared to native immunoglobulins. A toxicity study performed with homologous immunoglobulins in BALB/c mice demonstrated cationized homologous immunoglobulins have no tissue toxicity at a daily dose of 5 mg/kg. Immunocytochemistry showed that immunoglobulins were taken up into the intracellular compartment of human lymphocytes. Treatment of HIV-infected SCID mice that were transplanted with human lymphocytes demonstrated therapeutic efficacy at a dose of 5 mg/kg cationized human immunoglobulins. In conclusion, cationized immunoglobulins are potential antibody-based therapeutics for the treatment of viral diseases, such as AIDS; cationized antibodies undergo enhanced transport into brain and peripheral organs; when homologous cationized immunoglobulins are administered, there is no measurable tissue toxicity.

688.8

MCP-1 INDUCES TRANSMIGRATION OF PERIPHERAL BLOOD MONOCYTES ACROSS A MODEL OF THE HUMAN BLOOD-BRAIN BARRIER. S.A. Downie, J.W. Berman, K.M. Weidenheim and W.D. Lyman*. Pathology Dept., Albert Einstein Coll Med, Bronx, NY 10461.

A hallmark in the pathogenesis of central nervous system (CNS) inflammatory diseases is the presence of peripheral blood mononuclear cells (PBMC) in the CNS parenchyma. The signals and mechanisms contributing to extravasation of PBMC are as yet to be completely defined. One molecule suggested to have a role in this process is monocyte-chemoattractant peptide-1 (MCP-1). To investigate the potential role of this molecule in transmigration of PBMC, we used a model of the human blood-brain barrier (BBB) that is constructed with autologous human fetal endothelial cells and astrocytes. Freshly isolated human PBMC were labeled with rhodamine-conjugated dialkylcarbocyanine (DiI) and placed onto the endothelial (eg. luminal) surface of the model. MCP-1 (100 ng/ml) was infused into the medium surrounding the astrocyte (eg. abluminal) side. MCP-1 induced transmigration of PBMC was noted after 60 minutes of incubation and was maximal by 150 minutes. The ratio of transmigrated cells in MCP-1 treated cultures as compared with untreated controls was 14.5 at 150 minutes. This study supports the hypothesis that MCP-1 is involved in transmigration of inflammatory cells across the BBB and into the CNS parenchyma. This mechanism may contribute to the development of neuropathologies including that associated with AIDS.

(Supported in part by USPHS grants MH 47667 and MH 52974)

VISUAL CORTEX: STRIATE IX

689.1

PHYSIOLOGICAL CORRELATES OF ANISOTROPY IN HORIZONTAL CONNECTIONS: LENGTH SUMMATION PROPERTIES OF NEURONS IN LAYERS 2 AND 3 OF TREE SHREW STRIATE CORTEX. W. H. Bosking* and D .Fitzpatrick. Dept. of Neurobiology, Duke University Medical Center, Durham NC 27710.

Axon collaterals of layer 2/3 neurons form an extensive system of horizontal connections in tree shrew striate cortex (V1). These connections extend for several millimeters, linking neurons that have similar orientation preferences, but receptive fields separated by as much as 20 degrees of visual space. Axon collaterals of most layer 2/3 neurons are distributed anisotropically, extending farther and giving rise to more boutons along an axis in the map of visual space that corresponds to their preferred orientation (Fitzpatrick et al., '93).

We investigated the functional consequences of this precise modular and topographic specificity by making extracellular recordings from isolated single units in layer 2/3. For each unit, the classical receptive field (CRF) was determined using a minimum stimulation technique. Five to eight repetitions of stimuli consisting of bars of light between 1 and 40 deg in length were swept across the CRF at the preferred orientation. Most neurons (13/17) showed an increase in response to bars longer than the CRF. On average, a plateau in the length response curve was not reached until about 12 deg, even though the CRF for these cells was approximately 4 deg long. Some units (6/13) showed a progressive increase in response to stimuli as long as 40 deg. These data clearly indicate that the activity of cells in layer 2/3 of V1 can be modulated by visual stimulation outside the CRF. We also have evidence that in some cases, stimulation outside the CRF is sufficient to evoke responses from layer 2/3 neurons, independent of stimulation of the CRF. Supported by EYO6821.

689.2

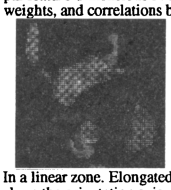
MEASURING THE DELAY OF THE ONSET OF EXTRA-RECEPTIVE FIELD MODULATION IN V1 NEURONS K. Zipser*. Dept. of Brain and Cognitive Sciences, MIT, E25-634, Cambridge, MA 02139

Given the same texture stimulation of the receptive field (RF), V1 neurons generally respond more vigorously when the pattern outside the RF differs from that inside the RF compared to the case where texture is of a homogeneous type across the display. We term this effect *extra-RF modulation*. Here we measure the delay of the onset of extra-RF modulation relative to the response latency of direct RF stimulation. Single and multi-unit recordings were made in area V1 of an awake, behaving monkey. As the monkey fixated in a behavioral trial, a given V1 neuron was initially stimulated with a homogeneously textured display flashed on a gray background. In some trials, after 150 ms of static texture stimulation, the texture outside the RF appeared to fall back in depth through stereoscopic disparity cues. In other trials, the homogeneously textured display remained unchanged. In all cases, the pattern over the RF itself remained the same throughout the texture display period. Compared to the case of the static homogeneously textured display, V1 neurons responded more vigorously following the change in the extra-RF texture pattern (i.e., the change in texture outside the RF caused extra-RF modulation during static RF stimulation). The delay of the onset of this extra-RF modulation was 30 to 50 ms relative to the normal response latency of the cells under study, or 80 to 100 ms relative to the change in texture outside the RF. This suggests that extra-RF modulation arises from reciprocal activity within the cortex rather than from direct feed-forward visual input. The delay in extra-RF modulation (which is lengthy compared to synaptic and conduction delays) raises the possibility that extra-RF modulation can reflect computationally complex analysis of visual input.

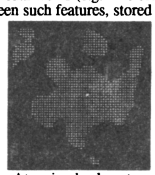
689.3

COOPERATIVE SELF-ORGANIZATION OF ORIENTATION MAPS AND LATERAL CONNECTIONS IN THE VISUAL CORTEX Joseph Sirosh* and Risto Miikkulainen, Dept of Computer Sciences, Univ. of Texas, Austin, TX 78712.

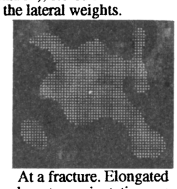
Recent experiments show that the lateral connections in the primary visual cortex self-organize from cortical activity, and that their patterns closely follow receptive field properties such as orientation preference. Through large-scale computer simulations, we show that a single Hebbian self-organizing process can account for the self-organization of oriented receptive fields, orientation maps, and the patterns of lateral connections. The model consists of an array of retinal receptors connected to an array of cortical neurons. Neighboring neurons are linked both by excitatory and inhibitory lateral connections, and farther neurons only by inhibitory connections. At each input presentation, neurons sum the input activity through the afferent connections, and repeatedly exchange activity through the lateral connections. When the activity settles, all connection weights are modified by the Hebbian rule. As training proceeds, the afferent connections organize into oriented receptive fields and orientation maps. The typical features of V1 maps, such as linear zones, pinwheels and fractures develop in the orientation map. At the same time, the long-range lateral connections learn the correlations in activity between cortical neurons and form patterns that closely follow the organization of the orientation map. The model permits us to predict the patterns of lateral connections at linear zones, pinwheels and fractures (*figures below*), and shows that the self-organizing process stores two kinds of visual knowledge in the cortex: the principal feature dimensions in the visual world (e.g. line orientation), stored in the afferent weights, and correlations between such features, stored in the lateral weights.



In a linear zone. Elongated along the orientation axis



At a pinwheel center. Links to all orientations



At a fracture. Elongated along two orientation axes

689.4

SINGLE-UNIT AND MULTI-UNIT MANIFESTATIONS OF LATERAL INTERACTIONS IN MONKEY VISUAL CORTEX. E. Katz*, K. P. Purpura and J. D. Victor. Dept. of Neurol. and Neurosci. Cornell Univ. Medical College, New York NY 10021.

Several lines of evidence indicate that inputs from beyond the classical receptive field play a role in shaping the responses of cortical neurons. Multi-input visual stimuli, such as those utilizing m-sequences (Sutter, 1987) are advantageous to studying these interactions, because these stimuli identify inputs to the receptive field that would be subthreshold if stimulated in isolation. With this technique we (Soc. Neurosci. 1993) demonstrated that the population receptive fields of local field potentials are modulated by a gain control driven by nearby visual input. We now extend these studies to single-unit and multi-unit recordings in macaque V1.

Lateral interactions of visual inputs were studied by comparing neural responses to contrast modulation of a 249-region stimulus (baseline condition) to responses to the same stimulus in which one or more of the regions were held at the mean luminance. This removal of modulation led to changes in the magnitude of the neural response to the regions which were within the receptive field. As we found in field-potential studies, changes in gain occurred within less then one minute, were readily reversible, and were not accompanied by a change in dynamics. However, an unexpected finding was that the effect of removal of modulation in one region was not always simply an overall increase in gain. Rather, in several cases, there appeared to be a selective augmentation of suppression of subregions within the receptive field. *Supported by EY9314 and The Revson Foundation.*

689.5

LONG RANGE LATERAL CONNECTIONS, NOT RECIPROCAL CIRCUITS, UNDERLIE HORIZONTAL PROPAGATION OF ACTIVITY: EVIDENCE FROM OPTICAL RECORDING OF FERRET VISUAL CORTEX SLICES.
D. A. Nelson* and L. C. Katz, Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

There is abundant evidence for rich lateral interactions in the visual cortex. In layer 2/3, lateral activation may be mediated by the clustered axon collaterals which extend from pyramidal neurons for a millimeter or more. Alternatively, polysynaptic, regenerative activation of neighboring columnar circuits extending across all cortical layers might mediate horizontal propagation. We tested these two models using optical recording and extracellular biocytin injections in coronal and tangential slices prepared from adult ferret visual cortex. Several lines of evidence support the former model. The rate of horizontal propagation in coronal slices (160 ± 9 mm/sec, n = 61) is consistent with a monosynaptic, not a polysynaptic circuit. Connections between layers are not required for horizontal propagation: in tangential slices containing only layer 2/3 neurons, the rate (130 ± 9 mm/sec) and extent (1200 ± 130 μm, n = 6) of horizontal propagation were similar to that in coronal slices. The pattern of activation in some tangential slices was patchy, as the tangential horizontal connection model predicts. Finally, horizontal propagation of activity in tangential slices was coextensive with biocytin labeled axon collaterals arising from the stimulus site (n = 4). In slices treated with bicuculline methiodide to remove inhibitory circuits, horizontal propagation of activity in layer 2/3 may occur via circuit elements other than long distance horizontal axons. Under this condition activity propagated at a much slower rate (coronal slice: 50 ± 7 mm/sec, n = 2; tangential slice: 45 ± 9 n = 3) and spread over a much greater extent (> 2200 μm). This implies that activity is epileptiform in such slices, and does not reflect normal circuit patterns. Thus, long distance horizontal connections play the central role in transmitting activity between cortical columns. Supported by NIH grant EY07690 (L.C.K.) and a Howard Hughes Predoctoral Fellowship (D.A.N.).

689.6

A CORTICAL ARCHITECTURE FOR REAL AND ILLUSORY CONTOUR PROCESSING IN AREAS V1 AND V2.
(W. D. Ross[1], S. Grossberg[2*], and E. Mingolla[3]) Cognitive and Neural Systems, Boston University, Boston, MA.

Neurophysiological data on global interactions in primary visual cortex (Gilbert & Wiesel 1990), sensitivity to illusory contours (Grosof *et al.* 1993) in V1 and data on long-range illusory contour sensitivity (von der Heydt & Peterhans 1989) in V2 can be explained by a single cortical architecture replicating similar interactions in V1 and V2 but at different scales. This system can also explain psychophysical data and clarifies how the cortex processes scenes degraded by noise, occlusion or camouflage. The model refines and simplifies the Boundary Contour System (BCS) for emergent boundary segmentation (Grossberg & Mingolla 1985). Cooperative lateral interactions act to strengthen contours and define context-sensitive completions and groupings. Competitive interactions sharpen boundaries across space and act to choose local boundary orientation and position based on global information. Lateral feedback across the cortical map naturally realizes these interactions. These mechanisms are replicated at both the model V1 and V2 stages. Simulations using a single set of parameters model physiological data on V1 and V2 responses to offset grating stimuli and psychophysical data on strength of illusory contours as a function of the number, density, and shape of inducers.

Gilbert, C. D. & Wiesel, T. N. (1990). *Vision. Res.*, **30**, 1689-1701.
Grosof, D. H., Shapley, R. M., & Hawken, M. J. (1993). *Nature*, **365**, 550.
von der Heydt, R. & Peterhans, E. (1989). *J. Neuroscience*, **9**, 1731-1748.
Grossberg, S. & Mingolla, E. (1985). *Percep. and Psychophys.*, **38**, 141-171.
[1]Supported by HNC SC-94-001. [2]Supported by AFOSR F49620-92-J-0499, ONR NOOO14-92-J-4015 & ONR N00014-91-J-4100.[3]Supported by AFOSR F49620-92-J-0334, NCEE A303-21-93 & ONR N00014-94-1-0597.

689.7

LASER PHOTSTIMULATION REVEALS CONVERGENCE OF MAGNO- AND PARVOCELLULAR INPUT ONTO INDIVIDUAL NEURONS IN LAYER 4B OF MACAQUE V1.
A. Sawatari and E.M. Callaway*, Neurosci. Pgm. and Dept. of Physiol., Univ. of CO Sch. of Med., Denver, CO 80262.

Magnocellular (M) recipient neurons in layer 4Cα of macaque V1 have strong axonal projections to layer 4B, while the parvocellular (P) recipient neurons in layer 4Cβ do not. These observations reinforce the long-held notion that layer 4B neurons receive most or all of their input from the M stream. This interpretation however, overlooks the possibility that layer 4B pyramidal neurons could receive input from layer 4Cβ onto their apical dendites in layer 3B.

We determined the locations of neurons that made functional connections with layer 4B neurons by recording monosynaptic excitatory postsynaptic currents (EPSCs) elicited by stimulation in other layers. Living coronal brain slices from V1 of macaque monkeys were bathed in ACSF containing "caged" glutamate, and whole-cell, voltage-clamp recordings were made from individual layer 4B neurons. Brief (5 msec) flashes of light from a UV laser, focused to a small spot by a microscope objective, were delivered to desired locations in the slice to uncage the glutamate and elicit action potentials in neurons with cell bodies near the stimulation site (within 50μm). Neurons were also intracellularly labeled with biocytin to allow later determination of axonal and dendritic morphology.

Our sample of layer 4B neurons includes 4 spiny stellates, 2 pyramidal cells, and 3 cells whose morphology could not be determined. As expected, EPSCs were frequently evoked in all layer 4B neurons following stimulation in 4Cα. However, for pyramidal neurons, EPSCs of similar magnitude were evoked just as frequently following stimulation in 4Cβ. EPSCs were also evoked in spiny stellates following stimulation in 4Cβ, but they were less frequent and their size was typically much smaller than those following stimulation in 4Cα. Following stimulation in layer 5, EPSCs were frequently evoked in pyramidal, but less often in spiny stellate cells, consistent with our observation that layer 5 pyramidal neurons project heavily to layer 2/3, but only weakly to 4B. Stimulation in layer 6 elicited EPSCs in all layer 4B neurons. This finding is consistent with our observation that layer 6 pyramidal neurons project heavily to 4B and 2/3. We conclude that layer 4B pyramidal neurons receive strong input, via 4Cα *and* 4Cβ, from both the M and P streams, but the input to spiny stellates is predominantly from the M stream, via 4Cα.

689.8

RELATIONSHIPS BETWEEN OCULAR DOMINANCE COLUMNS AND LOCAL PROJECTIONS OF LAYER 6 PYRAMIDAL NEURONS IN MACAQUE PRIMARY VISUAL CORTEX.
A.K. Wiser* and E.M. Callaway. Neurosci. Pgm. and Dept. of Physiol., Univ. of CO Sch. of Med., Denver, CO 80262.

To study the laminar and columnar specificity of axonal arbors from layer 6 pyramidal neurons in macaque V1, we intracellularly labeled neurons with biocytin in slices prepared from monkeys that had received an intraocular injection of tetrodotoxin (TTX) in one eye. The TTX injection decreases cytochrome oxidase (CO) expression in ocular dominance (OD) columns corresponding to the injected eye. Slices with labeled neurons were double-stained for biocytin and CO, to allow visualization of neuronal processes, as well as OD columns. We have analyzed 29 layer 6 pyramidal neurons from OD column-labeled slices.

We have previously defined 7 types of layer 6 pyramidal neurons. These types belong to 2 major classes. Class I neurons have dense axonal projections to layer 4C and lack dendritic branches in 5B. It has previously been suggested that the axonal projections of these cells might be specific for regions receiving input from a single eye in layer 4C (Anderson et al., 1993). Class II neurons have axonal arbors that are *not* focused on 4C, and numerous dendritic branches in 5B. Different types within each class are defined based on further laminar specificity of axonal and dendritic arbors. Type Iα cells have dense axonal projections (and dendritic branches) in layer 4Cα, type Iβ in 4Cβ, type IβA in 4Cβ and 4A, type Im in just the middle of 4C, and type IC throughout the depth of 4C. Class II neurons, although diverse, are not easily classified into distinct types, except for type IIB. Type IIB neurons have strong axonal projections to layers 4B, 2/3 and sometimes 5, but not 4C. They also have a dense dendritic arbor in layer 5 that does not extend above layer 5. The remaining class II neurons are designated as type II.

We labeled and analyzed 9 class I cells in TTX treated animals, including 4 type Iα, 1 type Iβ, 2 type IβA, and 2 type IC. No type Im cells were labeled. In addition 20 class II cells, including 3 type IIB, were labeled. None of the cells in our sample have axonal projections that are specific for columns dominated by one eye or the other. Instead the axonal arbors freely cross OD column borders, without any apparent change in the branching or density of projections. Some type II neurons have long, clustered projections within layer 6, but they are not OD column-specific.

689.9

ALTERATIONS IN STRIATE CORTICAL OCULAR DOMINANCE COLUMNS IN ANISOMETROPIC AMBLYOPIA.
A.W. Roe*[1], G.M. Ghose[1], E.L. Smith[2], Y.M. Chino[2], and D.Y. Ts'o[1]. Division of Neuroscience, Baylor College of Medicine[1], Houston, TX 77030 and College of Optometry, University of Houston[2], Houston, TX 77204.

Early unilateral defocus associated with anisometropic amblyopia produces deficits in binocular vision at high spatial frequencies and in the contrast sensitivity of the defocussed eye. These deficits are thought to reflect functional changes in the striate cortex as a result of competitive interactions between left- and right-eye inputs to individual neurons. To investigate the effects of this anomalous competition on cortical binocular organization, we examined the organization of V1 ocular dominance columns in amblyopes. Behaviorally confirmed, anisometropic amblyopia was produced in adult rhesus monkeys that were reared with a -10D lens in front of one eye. We used optical imaging techniques to visualize ocular dominance columns in the superficial layers of the parafoveal striate cortex near the V1-V2 border in response to different spatial frequency stimuli. We find that at high spatial frequencies (6-8c/deg), but not at lower spatial frequencies, the normal eye columns exhibit greater activation than amblyopic eye columns. Moreover, in comparison to normal monkeys, the ocular dominance columns in amblyopic monkeys have a greater spatial period and are more irregularly organized. Thus, in addition to alterations in the spatial frequency tuning characteristics of individual neurons, early unilateral optical defocus produces large-scale alterations in the binocular organization of the primate visual cortex which may contribute to the binocular vision deficits commonly exhibited by anisometropic amblyopes.
EY03611 (ES), EY08128 (YC), EY08240 (DT), EY06568 (GG).

689.10

Different Temporal Patterns of Immediate-early Gene mRNA and Protein Expression Allow Multiple Labeling Strategies in Visual Cortex of Monkey.
A. Chaudhuri[1], S. Larocque[1], J. Nissanov[2], L. Rioux[3]* & A. Goldszal[2]. [1]Dept. Psychology, McGill Univ., [2]Computer Vision Center for Vertebrate Brain Mapping, Drexel Univ. [3]Dept. Pharmacology, Univ. Pennsylvania.

It is now well established that neuronal activity produces transient changes in the expression of a number of immediate-early genes, such as *zif268* and *c-fos*. Previous studies have shown that immunodetection for their products may be used to label activated neurons under conditions of selective stimulation. We now report that the different time course of mRNA and protein induction, approximately 30 m and 2 h after onset of stimulation, may be used to visualize neurons that are separately activated under different stimulus conditions, thus allowing double labeling strategies.

We have used immunostaining to detect the Zif268 protein and in situ hybridization histochemistry (ISHH) to detect both *zif268* and *c-fos* mRNA in striate cortex of three adult vervet monkeys following a reverse-occlusion procedure. After three hours of monocular deprivation in chair-restrained awake animals, the patch was switched to the other eye for thirty minutes. An ocular dominance pattern was evident with both immunostaining and ISHH for *zif268*. However, the two patterns were clearly complementary; immunostaining revealed those columns that represented the open eye for the initial three hours whereas ISHH revealed columns that were activated during the final thirty minutes. Ocular dominance columns that were spatially aligned to the latter set were also visible with *c-fos* ISHH. However, it is unlikely that both labeled an identical set of neurons because Zif268 expression is known to be temporally correlated with ongoing activity whereas *c-fos* is induced in a transient, impulse-like manner. (Supported by MRC MA-12685 (AC); NIH award P41RR01638 (JN) & NIH award 1F32 NS09413-01A1 (LR)).

689.11

LUMINANCE CONTRAST GAIN, SPATIAL AND TEMPORAL FREQUENCY TUNING IN CO BLOBS AND INTERBLOBS OF MAMMALIAN STRIATE CORTEX MEASURED WITH INTRINSIC OPTICAL IMAGING. E.V. O'Brien*, R. Everson, and E. Kaplan. Laboratory of Biophysics, The Rockefeller University, N.Y., N.Y., 10021

The striate cortex of macaques contains regions of high metabolic activity, the cytochrome oxidase (CO) blobs. Some single unit studies report that their physiological properties are distinct from those of the surrounding regions, but other studies report no such differences. To circumvent the limitations of single unit studies, we used intrinsic optical imaging to compare the response of CO blobs to luminance contrast (LC), the tuning for spatial frequency (SF) and temporal frequency with those found in the interblob regions.

Reflectance changes from layers 2/3 of the exposed visual cortex of anesthetized and paralyzed monkeys and cats were imaged with a CCD camera in response to patterns which varied in luminance contrast, spatial frequency, or temporal frequency. The images were analyzed with extensions of the Karhunen-Loeve procedure (principal components analysis) and yielded spatio-temporal activity maps.

The contrast gain of the macaque cortex, measured optically, is similar to that of M and magnocellular neurons. The contrast gain of cat cortex is similar to that of single cortical neurons. We shall discuss the differences between the response properties of blobs and interblobs, and show that optical imaging of intrinsic signals can be used to study the LC sensitivity, SF and TF tuning in spatially localized regions of cortex, such as the blobs.

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689.12

THE ROLE OF THE ASCENDING SEROTONERGIC SYSTEM IN CORTICAL NITRIC OXIDE PRODUCTION. M.J. Friedlander*, F.W. Hester, C.D. Gancayco, B.D. Waterhouse† and R.C.S. Lin†. Neurobiology Research Center and Dept. of Physiology and Biophysics, Univ. of Alabama at Birmingham, 35294-0021 and †Dept. of Anatomy and Neurobiology, Hahnemann Univ., Philadelphia, PA 19102.

Nitric oxide (NO) production amplifies NMDA receptor-mediated glutamate release in the rodent cerebral cortex (Montague et al., Science, 263:973-977, 1994). Intrinsic nitric oxide synthase (NOS) or NADPH-diaphorase (NADPHd) positive neurons represent a small fraction (< 2%) of cortical neurons, yet the cortical neuropile is richly invested with NADPHd positive profiles. We investigated whether the ascending serotonergic pathways contribute to the NOS activity in the sensory neocortex of guinea pigs and rats. Cells of the dorsal raphe (DR) that project to the cortex were retrogradely labeled with fluorescent tracers and then stained for NADPHd and/or reacted immunohistochemically for serotonin (5-HT). Three sets of animals received intraperitoneal injections of the serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) or vehicle over two weeks at 75 µg/kg. They were evaluated for NADPHd histochemistry and the ability of cortical synaptosomal preparations to release endogenous glutamate in response to direct depolarization with 50 mM K^+ or to 100 µM N-methyl-D-aspartate (NMDA). We found that large numbers of 5-HT positive cells in the midline of DR that project to sensory cortical areas also are NADPHd positive and that 5,7-DHT treatment reduced NADPHd reactivity in the cortical neuropile, but did not reduce the number of intrinsic cortical NADPHd positive cells. Moreover, 5,7-DHT treatment reduced the NMDA receptor-NO-mediated release of endogenous glutamate by 40% relative to control without affecting direct depolarization-induced neurotransmitter release. Thus, the ascending serotonergic projection from the DR contributes significantly to the NO producing neuronal circuitry in neocortex.

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689.13

THE ROLE OF NITRIC OXIDE IN MODULATING THE VISUAL RESPONSE OF NEURONS IN CAT STRIATE CORTEX. P.Kara* & M.J. Friedlander. Neurobiology Research Center and Dept. of Physiology & Biophysics, Univ. of Alabama at Birmingham, 35294-0021.

Nitric oxide (NO) has been postulated to play a role in synaptic plasticity and development of the mammalian forebrain (Gally et al., PNAS. 87:3547-3551, 1990; Bohme et al., Eur. J. Pharmacol., 199:379-381, 1991). We recently demonstrated that NMDA receptor activation leads to NO production that enhances release of glutamate in synaptosomal preparations from the cerebral cortex (Montague et al., Science 263:973-977, 1994), and that NMDA receptor activation leads to potentiated synaptic transmission at excitatory synapses in cortical slice preparations (Harsanyi & Friedlander, Soc. Neurosci. Abstr., 21, 1995). In the present study, we have explored the role of NO in modifying visual responses in striate cortex, *in vivo*. Combined extracellular single-unit recordings and local micro-iontophoretic pharmacological manipulations were performed in 7 anesthetized and paralyzed cats. Three to five barrel micropipettes attached to tungsten-in-glass recording electrodes were used to deliver (a) nitric oxide synthase (NOS) inhibitors: L-nitro-arginine (LNA) or L-mono-methyl-arginine (LMMA), (b) their inactive D-enantiomers, and (c) the natural substrate for NOS: L-arginine. Thus, the endogenous NO turnover was manipulated while evaluating neural responses to drifting visual stimuli of optimal and sub-optimal configurations. For 15 of 22 neurons, NOS blockade (via application of 20-80 nA through the LNA or LMMA barrels) reduced the visual response and spontaneous activity by 20-80%. In 5 neurons, NOS blockade specifically facilitated the visual response by 40-100%. Equivalent iontophoretic currents through barrels containing D-forms of the NOS inhibitors were ineffective. These findings suggest that NO or its metabolites may play a role in signal amplification in the intact primary visual cortex.

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CALCIUM CHANNELS: PHYSIOLOGY, PHARMACOLOGY AND MODULATION II

690.1

VOLTAGE-DEPENDENT STATE TRANSITIONS OF SQUID NEURONAL CALCIUM CHANNELS. M.B. McFarlane* and W.F. Gilly. Departments of Molecular and Cellular Physiology and Biological Sciences, Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950.

We have studied the gating of fast-deactivating (FD) Ca channels of squid giant fiber lobe (GFL) neurons using whole-cell voltage clamp. Prolonged depolarization causes the appearance of a second open state of FD Ca channels (O_2) whose existence is observable as a difference in the rate of channel closure (measured from tail currents) that is roughly 2-fold slower than from the normal open state (O_1). Entry into O_2 follows an exponential time course (τ = 120 ms at +80 mV). Neither I_{Ca} amplitude of channels in O_2 nor the activation kinetics of I_{Ca} are significantly different from channels in O_1. FD Ca channels recover fast (O_1) closing kinetics within 50 ms at -80 mV (τ = 9-16 ms). Since this recovery is possible at potentials where channels are likely to be closed, channels in O_2 appear to open and close along a pathway independent from (but similar to) that leading to O_1. Further evidence for the $O_1 \rightarrow O_2$ transition is supported by the blocking effects of $NiCl_2$. Following prepulse depolarization, the level of Ni block increases as entry into O_2 progresses. Recovery ($O_2 \rightarrow O_1$) is observed at 0 mV in the presence of Ni, as the O_2-blocked level returns to that of O_1 (τ = 14 ms). These slower tail current kinetics would be observed as an increase in mean open time at -80 mV on the microscopic (single channel) level. Such behavior at negative potentials could lead to an increase in calcium influx at the motor terminal following repetitive giant axon stimulation.

690.2

NICKEL MODULATES TWO DISTINCT EFFECTS ON NEURONAL CALCIUM CHANNELS: BLOCK AND INHIBITION OF ACTIVATION-GATING. G.W. Zamponi, E. Bourinet, S.J. Dubel* and T.P. Snutch. Biotechnology Laboratory, Univ. of British Columbia, 237-6174 Univ. Blvd. Vancouver, B.C. Canada V6T 1Z3.

Nickel ions have been reported to exhibit differential effects on voltage-gated calcium channels. To obtain a more precise characterization of nickel action we have investigated the effects of nickel on four major classes of cloned neuronal calcium channels (α_{1A}, α_{1B}, α_{1C} and α_{1E}) transiently expressed in *Xenopus* oocytes. Nickel caused two major effects: 1) block of macroscopic currents and 2) a shift in the current-voltage relation towards more depolarized potentials which was paralleled by a decrease in the apparent number of gating charges. In 10 mM Ba, block followed 1:1 kinetics and was most pronounced for α_{1C}, followed by α_{1E}, α_{1A}, and α_{1B} channels. In contrast, the change in activation-gating was most dramatic with α_{1E}, with the remaining channel subtypes being less affected. The current-voltage shift was well described by a simple model in which nickel binding to a saturable site resulted in altered gating behaviour. Both the affinity for block and the shift in current-voltage were reduced upon increasing the external permeant ion concentration. Replacement of Ba with Ca reduced both the degree of nickel block and the effect on gating for α_{1A} and α_{1C} channels, but increased the nickel blocking affinity for α_{1E} channels. The coexpression of Ca channel β subunits was found to differentially influence nickel effects on α_{1A}: the β_{2a} and β_4 subunits resulted in larger current-voltage shifts compared to the β_{1b}, while elimination of the β subunit almost completely abolished the gating shifts. In contrast, block was similar for the three β subunits, while complete removal of the β subunit resulted in an increase in blocking affinity. Qualitative and quantitative differences in the dependence of the effects of nickel concentration on gating and block suggest two distinct nickel binding sites on neuronal calcium channels.

690.3

POLYAMINES INHIBIT VOLTAGE-GATED Ca^{2+} CHANNEL CURRENTS IN DORSAL ROOT GANGLION NEURONS AND IN OOCYTES EXPRESSING CLASS B Ca^{2+} CHANNELS. G.W. Campbell*[1], R.A. Gross[2] and D.M. Rock[1], [1]Neuroscience Therapeutics, Parke-Davis Research, 2800 Plymouth Rd., Ann Arbor MI 48105 and [2]Depts. of Neurology and Pharmacology, University of Rochester Medical School, 601 Elmwood Ave., Rochester, NY 14642.

Endogenous polyamines interact with a variety of ligand- and voltage-gated ion channels and may play a role as neurotransmitters or neuromodulators in the central nervous system (CNS). The effects of polyamines on glutamate receptors has been studied in detail, less is known about the interaction of polyamines with voltage-gated Ca^{2+} channels (Scott, RH et al. TINS, 16:153-159, 1993). Using whole-cell and two-electrode voltage-clamp techniques, we studied the effects of endogenous and structurally-related synthetic polyamines on Ca^{2+} channel currents in Dorsal Root Ganglion (DRG) neurons and in oocytes expressing Class B (N-type) Ca^{2+} channels.

High concentrations (100 μM - 10 mM) of the endogenous polyamines spermine (SP) and spermidine (SD) reduced the peak amplitude of high voltage activated Ca^{2+} currents in DRG neurons, with SP being more potent than SD. The synthetic polyamine arcaine inhibited DRG Ca^{2+} currents only at 10 mM. In preliminary experiments, SP (300 μM - 10 mM) , SD (1 and 3 mM) and long chain diamines (100 μM) reduced the peak amplitudes of expressed Class B Ca^{2+} currents, while equivalent concentrations of putrescine, cadaverine and diethylenetriamine did not affect peak current amplitudes.

These results suggest that high concentrations of released polyamines may have a presynaptic action, by affecting voltage-gated Ca^{2+} channels, as well as a postsynaptic action on both voltage- and ligand-gated channels to affect neurotransmission in the CNS.

This work was supported in part by NS 19613 (RAG).

690.4

FUNCTIONAL INTERACTION OF SYNTAXIN WITH N-TYPE AND Q-TYPE CALCIUM CHANNELS.

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Syntaxin is known to interact directly with N-type Ca channels (e.g. Bennett et al., Science **257**:255; Leveque et al., JBC **269**:6306; Horikawa et al., FEBS Let. **330**:236; Sheng et al., Neuron **13**:1303). A *Xenopus* oocyte expression system was utilized in order to investigate whether interaction with syntaxin has any effect on the functional properties of Ca channels. It was found that coexpression of N-type ($\alpha_{1B}\beta_3\alpha_2$) channels with syntaxin 1A promoted inactivation, causing a ~20 mV shift in the channel steady-state inactivation curve towards more negative holding potentials. The rate of channel recovery from inactivation was decreased 2-3 fold in the presence of syntaxin, also consistent with preferential binding of syntaxin to the inactivated state of the channels. The C-terminal (a.a. 168-288) but not N-terminal (a.a. 1-190) of syntaxin 1A influenced channel gating. When expressed in oocytes, Ca channels encoded by $\alpha_{1A}\beta_3\alpha_2$ (likely Q-type) were also affected by syntaxin in a similar way.

Since N-type and P/Q-type channels contribute jointly to transmitter release at nerve terminals, these data suggest that besides its role as a docking site for synaptic vesicles, syntaxin may also modulate presynaptic Ca channel activity.

690.5

CONCERTED ACTIONS OF MULTIPLE CALCIUM CHANNELS IN EXCITATORY SYNAPTIC TRANSMISSION. D.B. Wheeler*, A. Randall and R.W. Tsien. Department of Molecular and Cellular Physiology, Beckman Center B103, Stanford University School of Medicine, Stanford, CA 94305.

We have investigated the basis of the concerted actions of different Ca^{2+} channel subtypes in triggering neurotransmitter release at CA3-CA1 synapses of rat hippocampal slices. The K^+ channel blocker, 4-aminopyridine (4-AP), was used to modify the presynaptic action potential (AP) in hippocampal area CA1, elicited by field stimulation of the Schaffer collateral/commisural fiber bundle. Intracellular recordings with sharp electrodes showed that 100 μM 4-AP broadened the AP recorded in CA3 somata by slowing the repolarization phase. 4-AP caused a two-fold increase in the slope of the field excitatory postsynaptic potential (EPSP) and eliminated the dependence of synaptic transmission on N-type Ca^{2+} channels. The latter effect was reversed by lowering extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$). The effects of AP-broadening on Ca^{2+} influx through various channels were studied by application of mock APs to voltage-clamped, cultured cerebellar granule neurons. Slowing the repolarization phase of a mock AP increased current carried by N-, P/Q- and R-type channels to a similar extent. The sensitivity of the field EPSP to variations in $[Ca^{2+}]_o$ was studied in the absence and presence of 4-AP. The form of this relationship indicated that as Ca^{2+} influx was progressively increased, increases in synaptic strength would eventually approach saturation. The relative impact of spike broadening is greatly increased by the selective inhibition of either N- or Q-type channels. This is of potential physiological importance for circumstances where either type of channel is selectively down-modulated. Our findings are consistent with a simple scenario in which N- and Q-type channels both contribute Ca^{2+} influx to help trigger transmitter release in individual synaptic boutons. The dependence on multiple types of Ca^{2+} channels is not absolute, however, because simply increasing AP duration increases Ca^{2+} influx through both kinds of Ca^{2+} channels, thereby lessening the dependence of synaptic transmission upon any one subtype.

690.6

DIFFERENTIAL EXPRESSION OF P- AND Q-TYPE CALCIUM CURRENTS DURING EARLY DEVELOPMENT OF SENSORY NEURONS.

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Analysis of neuronal development has emphasized the importance of voltage-activated Ca^{2+} currents (I_{Ca}) during the initial period of differentiation. Whether the new identified non L, non N high-threshold calcium current subtypes are present in early embryonic neurons and developmentally regulated is still unknown. To resolve these issues, we used the whole-cell patch-clamp technique to record calcium current in dorsal root ganglion (DRG) neurons acutely dissociated from mouse embryos during development. We report dramatic in vivo changes in the expression of P-, Q- and R-type calcium currents between embryonic days-13 and -15, respectively before and after the period of target innervation. All three currents were distinguished on the basis of their sensitivity to AgaIVA. While R-type was stable over this developmental span, Q-type expression increased 2-3 fold and P-type current disappeared completely. These data show that P-, Q- and R-type calcium current are expressed by early embryonic DRG neurons, are developmentally regulated and are probably involved in specific key developmental events including natural neuron death and onset of synapse formation.

690.7

COORDINATION OF EXPRESSION OF N-TYPE CALCIUM CHANNEL SUBUNITS THROUGHOUT DEVELOPMENT. M.W.McEnery*, T.D. Copeland^, J. Striessnig#, H. Glossmann#, M. Pacioianu and C.M. Begg . Dept. of Physiology and Biophysics, Case Western Reserve University, School of Medicine, Cleveland, OH 44106, ^ABL-Basic Research Program, NCI-Frederick Cancer R & D Center, Frederick, MD 21702, and Dept. of Molecular Pharmacology, University of Innsbruck, Innsbruck, Austria.

The omega-conotoxin GVIA sensitive N-type voltage-dependent calcium channel (N-type VDCC) is implicated in many neuronal processes which include neurotransmitter release, neuronal migration, and synaptic vesicle docking. The N-type VDCC is comprised of several non-identical subunits as indicated by both biochemical studies (McEnery, et al., 1991; Witcher, et al., 1993 Leveque, et al., 1994) and molecular cloning. While there is little question that the alpha1 subunit protein of the N-type VDCC corresponds to the class alpha1B isoform, (Dubel, et al., 1992; Williams, et al., 1992), the identity of the beta subunit associated with the alpha1B subunit remains controversial. In addition, there is no information on the time course for the assembly of the N-type VDCC from component subunits. The purpose of this study is to examine the level of expression and assembly of alpha1B subunits and all beta subunits throughout development using a panel of anti-peptide, affinity purified antibodies to the rat alpha1B and beta subunits. The level of subunit expression throughout development when correlated with the density of [^{125}I]conotoxin binding sites may indicate the time course of subunit assembly and the acquisition of functional [^{125}I]CTX binding sites. [This work was supported in part by a grant from LHIMRF (MWM) and by the National Cancer Institute, DHHS under contract No. NO1-C0-74101 with ABL (TDC)].

690.8

LOCALIZATION OF PYRAMIDAL NEURONS THAT HAVE THE T-TYPE CALCIUM CURRENT IN GUINEA-PIG PREFRONTAL CORTEX. E. Geijo-Barrientos* and E. de la Peña. Instituto de Neurociencias y Departamento de Fisiología, Universidad de Alicante, 03080-Alicante, Spain.

One of the calcium currents present in cortical neurons is the low threshold calcium current or T type current, which contributes to the generation of rhythmic activity and bursts of action potentials. We have studied the distribution of the pyramidal neurons that have this current within guinea-pig prefrontal cortex. The experiments were done in coronal slices of 300μm of thickness that included the prefrontal cortex kept "in vitro" at 33-34 °C. Neurons were impaled with 3M potassium acetate filled electrodes to obtain recordings in current-clamp or single electrode voltage-clamp (SEVC), always in the presence of TTX (1μM) and TEA (10mM); the neuronal morphology was studied with intracellular staining with neurobiotin. In a sample of 125 regular spiking pyramidal neurons, 60% of the neurons had the T current, directly recorded under SEVC or recorded, in current clamp, as a calcium spike of 15-20 mV of amplitude activated from membrane potentials more negative than -70mV. The neurons which had the T current were localized in the mid-deep layers of the cortex, at more than 400μm from the pial surface and were absent in a band of 200 μm of thickness located just below layer I. Neurons that did not have the T current were found in all layers of the cortex (except in layer I) though slightly more frequently in superficial layers (between 200 and 600 μm from the cortical surface). These results provide a direct demonstration that low threshold calcium current is expressed in pyramidal neurons located in particular layers of prefrontal cortex.

Supported by Grant PB92-0347 from the DGICYT (Spain).

690.9

INOSITOLPHOSPHATES MODULATE LIPID BILAYER RECONSTITUTED CALCIUM CHANNEL ACTIVITY. B.D. Cherksey*, M. Sugimori and R. Llinás, Dept. Physiology & Neuroscience, New York University Medical Center, 550 First Avenue, New York, NY 10016.

The effects of the phosphoinositides on P-type calcium channel activity was studied in cerebellar membrane vesicles and on the isolated P-channel protein using the "tip-dip" bilayer technique. Cerebellar membranes were obtained from freshly dissected cerebella. The membrane vesicle preparation was implemented in 400 mM sucrose. Electrical activity was measured in solutions containing 80 mM $BaCl_2$, 10 mM HEPES, pH 7.4 in the bath; 120 mM CsCl, 10 mM HEPES, pH 7.4 in the pipette.

P-channel protein was obtained using the polyamine, 1-arginyl N,N',-Bis(3-aminopropyl)-1,4-butane-diamine, which we have previously termed synthetic sFTX(3:4) coupled to Sepharose 4B CL via a 1,4-Butanediol Diglycidol ether linkage. This affinity gel allowed the extraction of a protein from bovine cerebellum homogenate. The electrical activity was measured in solutions containing: 80 mM BaCl2, 10 mM HEPES, pH 7.4 in the pipette; 120 mM CsCl, 10 mM HEPES, pH 7.4 in the bath.

To study the effect of phosphoinosides on electrical activity from membrane vesicles, the compounds were added to the solution in the pipette (cytoplasmic face of the channel) in concentrations ranging from 0.1 uM to 1.0 uM. IP3, at concentrations greater than 0.3 uM was found to produce an increase in the number of electrical events at any holding potential without an increase in the average open time per event. The electrical activity was blocked by the addition of sFTX to the bath. Neither PIP nor IP4 produced an activation of the P-channel nor did the synthetic stereoisomer of natural IP3. IP3 was without effect when applied to the bath (extracellular face of the channel).

Studies of the phosphoinositides on the isolated, reconstituted P-channel produced an identical pattern of results. The activation of the P-channel was prevented by the addition of heparin at a concentration of 100 µg/ml.

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TRANSPLANTATION II

691.1

DOPAMINERGIC RESPONSES TO STRIATAL INJURY. D.W.Howells*, G.T.Liberatore, J.Y.F.Wong, G.A.Donnan. University of Melbourne, Dept. of Medicine, Austin and Repatriation Medical Centre, Heidelberg, VIC 3084, Australia.

A potential therapy for Parkinson's disease lies in the stimulation and control of neurite outgrowth from nigrostriatal dopaminergic neurones. We have previously shown that the injury associated with implantation of gel foam to normal mouse striatum increases binding of [^{3}H]-mazindol to presynaptic dopamine uptake sites 10 months after implantation ($P<0.01$). When combined with the observation by others of increased density of host tyrosine hydroxylase immunoreactive fibres after striatal grafting or cavitation, it seems likely that striatal injury is stimulating proliferation and sprouting of dopaminergic terminals. We have now shown that striatal cavitation induces a concerted response from the dopaminergic system with proliferation of striatal presynaptic dopamine uptake sites ($P<0.05$), increased tyrosine hydroxylase activity ($P<0.05$), and increased concentrations of dopamine ($P<0.05$), dihydroxyphenylacetic acid ($P<0.05$) and homovanillic acid ($P<0.05$). Interestingly, while the changes of presynaptic dopamine uptake site density are only seen ipsilateral to the striatal lesion, the changes in tyrosine hydroxylase activity, dihydroxyphenylacetic acid and homovanillic acid concentrations are bilateral. These presynaptic changes are accompanied by decreases of the predominantly postsynaptic binding of [^{3}H]-SCH23390 and [^{3}H]-sulpiride to the D_1 and D_2 classes of dopamine receptors in the striatum. The density of presynaptic dopamine uptake sites, D_1 and D_2 receptors in the substantia nigra does not change after striatal injury. The striatal response to injury first becomes apparent 2 weeks after cavitation and gradually increases with time, resulting in a doubling of the DOPAC concentration and presynaptic dopamine uptake site density within 2 and 10 months respectively. If similar changes occur after intrastriatal transplantation in human Parkinsonism, the time frame and extent of the host dopaminergic responses would suggest that they may make a significant contribution to any observed clinical improvements.

691.2

XENOTRANSPLANTATION AND ANTIGEN MASKING OF FETAL PORCINE VENTRAL MESENCEPHALON IN A RAT MODEL OF PARKINSON'S DISEASE. W.R. Galpern*[1,3], L.H. Burns[1], T.W. Deacon[1], J. Dinsmore[4], and O. Isacson[1,2] [1]Neuroregeneration Laboratory, McLean Hospital, Belmont, MA, 02178; [2]Department of Neurology and Program in Neuroscience, Harvard Medical School, Boston, MA, 02114; [3]University of Massachusetts Medical Center, Worcester, MA, 01655; [4]Diacrin, Inc., Charlestown, MA, 02129.

The practical and ethical limitations inherent to current human neurotransplantation protocols suggest the potential utility of developing alternate cell sources. Fetal porcine neuronal xenografts can survive in and functionally reinnervate the host CNS using conventional immunosuppression. Xenotransplantation of fetal porcine neuroblasts pretreated with antigen binding fragments [$F(ab')_2$] of a monoclonal antibody to porcine major histocompatibility complex (MHC) class I may provide an adjunct or alternative to current immunosuppression regimens. We have recently shown that $F(ab')_2$ pretreatment enhances striatal xenograft survival in a rat model of Huntington's disease (Pakzaban, et al., Neuroscience, '95, **65**, 983-996). In order to evaluate the immunoprotective efficacy of $F(ab')_2$ masking of porcine MHC I in the 6-hydroxydopamine (6-OHDA) model of Parkinson's disease, rats were transplanted with fetal porcine ventral mesencephalon cell suspension into the denervated striatum and received either A) daily cyclosporine (10 mg/kg, sc), B) $F(ab')_2$-pretreated cells, or C) no immunosuppression. Behavioral recovery was monitored by amphetamine-induced rotation at approximately 6 week intervals following transplantation. Rats were sacrificed at 19 weeks post-transplant, and xenograft survival and axonal growth were assessed by tyrosine hydroxylase (TH) and donor-specific glial immunostaining. TH-positive neuronal survival was greater in the cyclosporine group than in either the $F(ab')_2$ or no immunosuppression groups. Ongoing studies are aimed at evaluating the xenograft rejection process associated with cyclosporine discontinuation and determining whether $F(ab')_2$ treatment is able to induce tolerance and prevent graft rejection following removal of cyclosporine immunosuppression.

691.3

FDOPA-PET STUDIES IN THE VERVET MONKEY: PRE-TRANSPLANT ASSESSMENT OF MPTP-LESION STABILITY. W.P. Melega*, M.J. Raleigh, D.B. Stout, A.A. DeSalles, S.R. Cherry, S-C. Huang, M.E. Phelps. Molecular and Medical Pharmacology, UCLA Sch. of Med. Los Angeles, CA 90095

Biochemical determination of the stability of MPTP-induced striatal dopamine lesions in monkeys can be achieved only with noninvasive imaging techniques. With PET, individual subjects can be scanned serially at pre-MPTP, and throughout the post-MPTP time period for quantitation of the extent and stability of the MPTP lesion over time. Unilateral intracarotid MPTP administration allows for PET measures of ipsilateral (lesioned) striatum to be evaluated relative to the contralateral (unlesioned) striatum. This paradigm significantly reduces the variance associated with interstudy comparisons. For these studies, parametric images of the FDOPA uptake rate constants (Ki) were generated by Patlak analysis. ROIs drawn on each control were transferred onto its corresponding post-drug image set. Pre-MPTP scans in adult male vervet monkeys (Cercopithecus aethiops sabaeus; n = 8) showed right/left striatal Ki ratios between 0.95 and 1.05. For the MPTP studies, subjects (n = 5) received [18F]fluoro-L-DOPA (FDOPA)-PET scans prior to MPTP injection and at post-MPTP times: 1-2, 5-7 and 9-11 months. FDOPA plasma metabolite profiles were similar to controls. For four of five subjects, striatal Ki ratios varied less than 5% between scans. Differences between subjects were still detected: right/left striatal Ki ratios were between 0.10 and 0.28. One subject showed stable post-MPTP ratios up to 7 months (0.12) but then showed partial recovery at 9 months (0.29). The results indicate that individual variability-reaction to MPTP occurs and that stability assessment can be obtained with FDOPA-PET. These measures provide a baseline for accurate longitudinal assessment of post-intervention (e.g. fetal transplant) strategies in this Parkinsonian model.

691.4

EMBRYONIC DOPAMINE CELL IMPLANTS IN HUMANS WITH ADVANCED PARKINSON'S DISEASE: RESULTS FROM 7 MONTHS TO 7 YEARS AFTER TRANSPLANT IN 23 PATIENTS. C.R. Freed , R.E. Breeze, S.A. Schneck, M. Leehey, C.F. O'Brien, L. Thompson, L.O. Ramig, F.G. Kaddis*, J.C. Mazziotta[1], D. Eidelberg[2], and A.A. Ansari[3], Univ. of Colorado Sch. of Med., Denver, CO 80262; UCLA Sch. of Med., Los Angeles, CA 90024[1]; North Shore University Hospital, Manhasset, NY 11030[2]; and Emory Univ. Sch. of Med., Atlanta, GA 30322[3].

Embryonic dopamine cell transplants are an evolving therapy for patients with advanced Parkinson's disease. Since 1988, we have performed 23 transplants in patients. Two were unilateral in caudate and putamen and 21 were bilateral into putamen using up to 16 needle passes. Only tissue from embryos 7 to 8 weeks post conception was used. Tissue from a single embryo was used in 6, from 2 embryos in 13, and 4 embryos in 4 patients. Six of the first 12 patients were immunosuppressed. Clinical change was monitored by UPDRS and ADL as well as frequent videotaping and computer testing in the home before and after first daily doses of drugs. PET scans were performed when available. Overall results showed 1) "On" and "off" performance improved three to twelve months after transplant. 2) Immunosuppression offered no apparent benefit. 3) Transplant growth and clinical improvement progressed up to four years after transplant. 4) Drug doses were reduced in most patients by about 50%. 5) Reduced severity of both "on" and "off" phases and improvement in sleep were commonly noted. 6) There was no improvement in memory or other cognitive tests, and patients with memory disturbance prior to surgery were made worse for many months after surgery. 7) Complications included an intraoperative hemorrhage leading to death one month later, a cortical stroke remote from the transplant site in a patient with cerebrovascular disease, and a small putamenal bleed in one needle tract. 8) Surviving tyrosine hydroxylase positive cells were seen in the patient who died. Further evidence for transplant survival was seen on fluorodopa PET scans.

691.5

A NEW FRONTAL APPROACH TO EMBRYONIC NEURAL TRANSPLANTATION IN HUMANS WITH ADVANCED PARKINSON'S DISEASE. R.E. Breeze , C.R. Freed*, S.A. Schneck, M. Leehey, and C.F. O'Brien, Univ. of Colorado Sch. of Med., Denver, CO 80262.

Since 1988, we have used closely spaced needle tracts to deposit embryonic dopamine cells in the putamen in patients with advanced Parkinson's disease. This approach through bilateral craniotomies at the apex of the skull over frontal cortex required up to 16 needle passes. We had estimated that the risk of serious hemorrhage was about 1/500 needle passes or less. This technique has been adopted by other groups performing neurotransplants. Our 17th patient had a catastrophic hemorrhage (after about 300 needle passes had been made into patients under this protocol). To reduce surgical morbidity, we devised a new transplant strategy entering the forehead via twist drill holes. Four needle tracts are used to deposit 30 mm cores of tissue along the long axis of putamen. Tissue from 4 embryos was used in four patients and two embryos in two patients. Six patients have undergone this procedure and are 8 to 18 months post surgery. Surgical time was cut from 4 hours to 2 hours. Hospitalization and postoperative headache were reduced. 3/6 patients were discharged the day after surgery. Results parallel those seen in the total population of 23 patients on whom we have performed transplants. For the first 3 patients, Activities of Daily Living Score fell from 23 to 10 in the "off" phase and 8 to 4 in the "on" phase by 7 to 9 months after surgery. Drug doses were reduced by up to 50%. These results show that a frontal approach offers good transplant outcome with apparently less surgical morbidity.

691.6

THE USE OF A COSMETIC SURGICAL PLACEBO CONTROLLED TRIAL IN THE TREATMENT OF PARKINSON'S DISEASE. T.B. Freeman*, D. Vawter, C.G. Goetz, R.A. Hauser, J.H. Kordower, P.R. Sanberg, B.J. Snow and C.W. Olanow. University of South Florida Div. of Neurosurgery, Tampa Florida.

Prospective randomized double-blind placebo-controlled trials are currently utilized for evaluation of new drugs. However, clinical trials investigating new surgical procedures have yet to widely include either cosmetic surgical placebo controls ("imitation operations") or sham surgery in the experimental design.

Our NIH-sponsored evaluation of fetal nigral tissue transplantation in Parkinson's disease includes a cosmetic surgical placebo control arm. The rationale for this design will be discussed including: 1) delineation of the difference between a cosmetic surgical placebo and sham surgery; 2) examples of detrimental noncontrolled surgical trials; 3) examination of costs and morbidity due to unnecessary surgery in the absence of placebo controlled trials; 4) discussion of medical placebo controlled trials where risk is greater than in surgical placebo controlled trials; 5) review of precedents for surgical placebo controlled trials; 6) identification of criteria for beginning randomized surgical placebo controlled trials; and 7) evaluation of the use of cosmetic placebo controlled trials in transplant protocols for Parkinson's disease.

A cosmetic surgical placebo controlled trial is ethical and appropriate at this stage of scientific knowledge, and necessary in order to accurately evaluate efficacy of neural transplantation in the treatment of Parkinson's disease.

691.7

GROWTH, DIFFERENTIATION, AND BLOOD-BRAIN-BARRIER OF MURINE EMBRYONIC CNS TRANSPLANTS. Stefan Isenmann*, Sebastian Brandner, Guido Kühne[1], Ulrich Sure, and Adriano Aguzzi. Institute of Neuropathology, University Hospital, Zürich, Switzerland. [1]Paul-Scherrer-Institute, Villigen, Switzerland.

Telencephalic grafting of embryonic tissue represents a powerful tool for developmental studies. We have recently used this technique to study long term effects of various lethal mouse mutants *in vivo*. However, a full exploitation of the grafting approach requires a thorough knowledge of the biological behavior of grafted tissue.

We have characterized the biological properties of embryonic telencephalic tissue isolated at different developmental stages and grafted into the caudoputamen and lateral ventricles of histocompatible mice. 164 grafts were analyzed up to 500 days after transplantation. Grafted cells were identified by autoradiography to ^{3}H-thymidine. Proliferation was examined by immunocytochemistry to 5-bromo-2'-deoxyuridine (BrdU). The graff size was measured as a function of the embryonic age of the donor tissue at transplantation. The time course of blood-brain-barrier reconstitution was examined *in vivo* by contrast enhanced magnetic resonance imaging (MRI) and we compared the results with various histological techniques in individual grafts.

Transplantation of embryonic telencephalic tissue resulted in the formation of solid neural grafts in 79% of all transplants. Our data indicate that the optimal time point for grafting murine telencephalic tissue is embryonic day E 12.5. Grafts transplanted at this stage resulted in 87% takes and grew larger and more reproducibly than tissue obtained at earlier or later stages. Proliferative activity was initially brisk (up to 35%), but ceased during the third week after transplantation. The blood-brain-barrier was shown to be reconstituted in most cases 7 weeks after transplantation. These data on the biology of wild type grafts provide important baseline information for studying grafts of "knock out" and transgenic tissue of embryonic lethal phenotypes.

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691.8

GRAFT-INDUCED RESTORATION OF FUNCTION IN HEREDITARY CEREBELLAR ATAXIA. L.C. Triarhou*, W. Zhang, W.-H. Lee. *Dept. Pathol. Lab. Med. & Pediatrics, Indiana Univ. Sch. of Med., Indianapolis, IN 46202-5120*

We have been using *pcd* mutant mice, a model of recessively inherited cerebello-olivary atrophy, to study the structural integration of transplanted wild-type Purkinje cells into the disrupted cerebellar loop (*Ann. Neurol 20:* 138, 1986; *Anat. Embryol. 176:* 145, 1987; *Neurochem. Res. 17:* 475, 1991). The aim of the present study was to determine the recovery of behavioral responses after bilateral grafting of E12 cerebellar cell suspensions into the deep cerebellar nuclei of the hosts, according to a protocol that emphasizes reconstruction of the missing cortico-nuclear projection (*Anat. Embryol. 185:* 409, 1992). Motor coordination and fatigue resistance were assessed in a rota-rod treadmill apparatus for mice. Mutants of the sham-operated group (n=6) stayed on the rotating drum for an average of 3.3 sec preoperatively and 3.0 sec postoperatively; *pcd* mice in the transplant-receiving group (n=6) stayed on the drum for 3.8 sec preoperatively and 13.5 sec postoperatively; the graft-induced 255% improvement was statistically significant at P=0.027. Moreover, grafted mice were able to sustain the abdomen raised above the ground on their limbs during movement, contrasting to the typical lowered, widened stance of *pcd* mutants. The viability of transplanted Purkinje cells was verified with immunocytochemistry for calbindin-D_{28k} and $GluR_{2/3}$ AMPA receptor subunits. An axonal innervation was supplied by donor Purkinje cells to the deep nuclear complex of the host. Further, most of the transplanted Purkinje cells had migrated to occupy cortical localities, and displayed a correct orientation of their dendritic trees toward the pia. Our findings provide the first demonstration of motor enhancement in a model of inherited cerebellar ataxia after intraparenchymal grafting of fetal cerebellar neurons. (Supported in part by USPHS grant award R29-NS29283).

691.9

FATES OF CORTICAL AND CEREBELLAR PROGENITOR CELLS TRANSPLANTED INTO THE NEONATAL SUBVENTRICULAR ZONE. T. Zigova*[1], R. Betarbet[1,2], R.A.E. Bakay[2] and M.B. Luskin[1]. [1]Depts. of Anat. and Cell Biology & [2] Neurosurgery, Emory Univ. School of Medicine , Atlanta, GA 30322.

Our studies have shown that cells derived from the anterior part of the neonatal subventricular zone (SVZa) are destined to become interneurons of the olfactory bulb (OB). The migration of SVZa-derived neurons to the OB does not appear to be guided by radial glia, although SVZa-derived neurons traverse a stereotypical path to the middle of the OB. Upon reaching the OB they ascend radially into one of overlying cellular layers. Recently, we have demonstrated that homotopically transplanted SVZa-derived cells adopt a spatio-temporal pattern of migration that is indistinguishable from that ordinarily used by SVZa-derived neurons. Here we investigated whether newly-generated neurons, which usually migrate along radial glia, can navigate the highly restricted path adhered to by SVZa-derived cells. Dissociated cells from the ventricular zone (VZ) of the embryonic day 16 or 17 rat telencephalon or from the external granule cell layer (egl) of the postnatal day 5 (P5) or P6 cerebellum, were labeled with either the cell proliferation marker BrdU or the fluorescent lipophilic dye PHK26 and stereotaxically implanted into the SVZa of P0-P2 rats. We found that heterotopically engrafted VZ cells, remained at the site of injection in the host SVZa. In contrast, heterotopically transplanted egl cells traversed the migratory pathway, although most did not migrate away from the middle of the OB. These results suggest that cells of different origins have varying abilities to decipher the guidance cues associated with the migratory pathway of SVZa-derived cells. Supported by NIH and March of Dimes.

691.10

MAINTAINED NEUROTROPIC SPECIFICITY IN RECONSTRUCTION OF THE ADULT CNS BY NEURAL TRANSPLANTS O. Isacson*, T.W. Deacon, W.R. Galpern, L.H. Burns, I. Dinsmore[1], P. Pakzaban Neuroregeneration Laboratory, McLean Hospital, Belmont, MA 02178, Department of Neurology and Program in Neuroscience, Harvard Medical School and (1) Diacrin, Charlestown, MA.

Neuron-target interactions during normal development of the mammalian CNS follow phenotypic and species determined cellular schedules. This order may suggest that developmental timing is essential for correct growth of the maturing CNS. To determine the capacity of fetal neural tissue to interact with the adult CNS in heterochronic circuitry reconstruction, fetal porcine striatal (LGE) or ventral mesencephalic (VM) cells were transplanted into ectopic or homotopic sites of immunosuppressed adult rats. Grafts developed and stained for a pig-specific neurofilament marker (70 kD neurofilament; NF70), a pig-specific astroglial cell marker (CD44) and other graft and host specific markers. LGE cell implants placed in the adult lesioned striatum grew axons into white-matter tracts with anatomically appropriate projections into gray matter striatal target-zones, globus pallidus, entopeduncular nucleus and substantia nigra pars reticulata. CD44-immunoreactive graft glial cells extended long fibers into host white matter tracts, but not into gray matter target-zones. VM grafts placed ectopically in striatum grew TH+ dopaminergic axons directly into striatal gray matter but not into white matter tracts. Conversely, NF70 staining demonstrated that non-dopaminergic neurons from such VM cell grafts projected specifically to gray matter zones of thalamic ventral anterior/ventral lateral, centrolateral , parafascicular and mediodorsal nuclei, consistent with projection patterns of neuronal phenotypes normally found in substantia nigra pars reticulata and deep mesencephalic nuclei. Axons from fetal VM cells placed in mesencephalon also projected to these host targets. Our findings indicate that neurotropic signalling for anatomic specificity seen during development is maintained over long-distances also in the mature CNS.

691.11

NEUROTROPHIN AND TRK RECEPTOR UPREGULATION IN REGIONS OF TARGETED NEOCORTICAL CELL DEATH COINCIDES WITH DIRECTED MIGRATION AND DIFFERENTIATION OF TRANSPLANTED NEURAL PRECURSORS. Y. Z. Wang* and J. D. Macklis. Dept. of Neurology & Prog. in Neuroscience, Harvard Medical School, Children's Hospital, Boston, MA, 02115

Transplanted embryonic neocortical neurons and clonal, multipotent neural precursors undergo directed migration and differentiation that is sharply limited to regions of targeted photolysis in mice, in which pyramidal neurons undergo apoptotic degeneration. To investigate the mechanisms of microenvironmental alteration underlying this directed migration and differentiation that occurs approximately 4-21 days after initiation of neuronal degeneration, we examined the gene expression of neurotrophic factors and trk receptors in geographically defined regions undergoing targeted apoptosis. We compared these regions to control regions in intact mice and to control regions adjacent to the targeted areas. Pyramidal neurons in C57B/6J mice were retrogradely targeted with photoactive nanospheres and noninvasively activated at 4 weeks of age to initiate apoptotic degeneration in lamina II/III of neocortex. We examined the experimental and control regions for mRNA levels of NGF, BDNF, NT-3, NT-4/5 and trkA, B, C by Northern blot analysis. BDNF, NT-3, NT-4/5 and trkB were specifically induced over the period of ongoing apoptosis in the experimental regions. TrkB mRNA increased earliest, with a maximum at 3 days and reduction rapidly after 6 days. Induction of BDNF mRNA began at 1 day with maximal induction at 6 days, then gradual decrease to control levels 21 days after initiation of cell death. NT-3 and NT-4/5 mRNAs were upregulated between 1 and 8 days after initiation. NGF and trkA, C mRNAs were not altered. Control regions from intact and experimental mice showed no mRNA upregulation. These findings suggest that the induction of BDNF, NT-3, NT-4/5 and trkB mRNAs are specific molecular effects of targeted apoptosis that may partially underlie developmentally appropriate cellular behavior in regions of photolytic degeneration in adult mice. Further characterization and cellular localization may provide insight regarding signaling pathways responsible for neuronal migration and differentiation during development, and toward potentially therapeutic neural precursor transplantation.

691.12

EVIDENCE SUGGESTING MIGRATION OF HOST NEURONS INTO FETAL ANTERIOR HYPOTHALAMIC GRAFTS. M.N. Lehman', J. LeSauter, C. Kim, and R. Silver. Dept. Cell Biol., Neurobiol. & Anat., Univ. Cincinnati Coll. Med., Cincinnati, OH 45267; Dept. Psychol., Barnard Coll., New York, NY 10027.

Recent work has demonstrated that migration of neuronal precursors continues in the adult mammalian brain (Lois & Alvarez-Buylla, Science, 264:1145, 1994). While studying intraventricular fetal anterior hypothalamic (AH) grafts, we have found surprising evidence suggesting that a subset of adult host neurons, or their progenitors, migrate into these grafts. Fetal (E15) hamster AH tissue was implanted into the third ventricle of immunosuppressed adult, SCN-intact rats (n=9). Graft recipients were perfused after 2 weeks and every fourth brain section (50 μm) was immunostained using a neurofilament (NF) antibody (RMO 108, gift of Dr. V. Lee) that recognizes rat but not hamster NF. AH grafts in 6 of 9 recipients were robust and viable, and 5 of these showed substantial fiber ingrowth from the host, specifically from attachment sites along the dorsal and lateral edges of the third ventricle. In addition, within each of these grafts, large (20-30 μm soma diameter) neurons of presumptive host origin were seen in association with host fibers. Sections from each graft contained between 3-15 host neurons; some cells were located 250-300 μm from the graft-host border. The migration of adult neurons, or their precursors, into fetal grafts may represent a previously unsuspected aspect of potential plasticity in the adult brain. Whether this is dependent on the source of fetal tissue or its site of implantation is not known. Experiments are underway to determine whether this phenomenon is unique to xenografts, perhaps reflecting the absence of species-specific inhibitory signals, or a general feature of homografts as well. Supported by NIH NS28175 (MNL) and NS 24292 (RS).

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS V

692.1

CORTICAL CONNECTIONS FROM PHYSIOLOGICALLY DEFINED NUCLEI OF THE SOMATOSENSORY THALAMUS OF MACAQUE MONKEYS. L.A. Krubitzer[1]*, S.L. Florence[2], N. Jain[2], and J.H. Kaas[2] [1]University of Queensland, Australia; [1]University of California, Davis, CA; [2]Vanderbilt University, Nashville, TN.

We examined the topographic organization of thalamic nuclei that project to somatosensory cortical fields in macaque monkeys. Electrophysiologically identified representations in anterior parietal fields 3a, 3b (SI), 1 and 2 were injected with retrograde tracers, then at the time of sacrifice the somatosensory thalamus was mapped using multiunit recording techniques. Tracers were placed either into representations of the same body part in different cortical fields or into different body part representations of the same cortical field. Results indicate that the ventral posterior nucleus, ventral posterior inferior nucleus, ventral posterior superior nucleus and ventral lateral nucleus contain separate complete representations of the body surface, and are distinguished by different response properties, stimulus preferences, architecture, and thalamocortical connections. Projections from the thalamus to electrophysiologically identified representations in cortex were both divergent and convergent, and projections to body part representations within a cortical field were not strictly homotopic. Such heterogeneous projections may provide the anatomical substrate for somatosensory cortical plasticity in adult primates after peripheral denervations.

692.2

HUMAN THALAMIC NEURONS RECEIVE SUBLIMINAL INPUT FROM REGIONS THAT ARE ADJACENT TO THEIR NATURAL MECHANICAL RECEPTIVE FIELDS. Z.H.T. Kiss*, K.D. Davis, R.R. Tasker, A.M. Lozano, J.O. Dostrovsky. Departments of Surgery and Physiology, University of Toronto, Toronto, Ontario, Canada.

Acute deafferentation has been shown to cause rapid alterations in neuronal receptive fields (RFs) in the human thalamus. The RFs of tactile neurons tend to shift/expand into regions immediately adjacent to those of the original RFs, when input from the original RF is eliminated. The aim of this study was to test the hypothesis that thalamic neurons receive subliminal inputs from regions adjacent to their receptive fields, as such inputs might form the basis for the deafferentation-induced effects.

During stereotactic thalamic exploration in patients being treated for pain or tremor, microelectrode recording identified neurons responsive to mechanical stimuli delivered to small RFs on the hand. In 10 patients, the effects of electrical stimulation (ES; bi- or monopolar, 0.5 ms pulse width, 1-10 Hz) delivered within and outside the RF was examined. Eleven of 23 cells were excited by ES within the RF and post-stimulus time histograms demonstrated a latency to activation of 22.3±2.5 ms (mean±SD). Of the 51 sites tested with ES outside the RF, 8 cells at 14 different sites responded with a latency of 40.8±14.5 ms. ES both within and outside the RFs was also found to produce inhibition 22-57 ms after the ES.

Latency of activation of human Vc neurons from outside the RF is significantly longer ($p<0.02$) than latency from within the RF, suggesting mediation by indirect polysynaptic pathways. These data also constitute the first direct demonstration of inhibitory inputs to human Vc tactile neurons. The results suggest that subliminal inputs may be important in mediating thalamic and cortical plasticity.
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692.3

OCCUPATIONAL HAND CRAMPS GENESIS PARALLED BY DEGRADATION OF REPRESENTATION IN THE SI CORTEX
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Repetitive, sustained gripping under conditions of high force, vibration and/or end range motion is associated with a task specific loss of voluntary control referred to as occupational hand cramps or focal dystonia of the hand. Etiology of this disorder is poorly understood; treatment success is limited. This study was designed to measure behavioral and neurological consequences of repetitive, sustained gripping with a vibrotactile stimulus. Adult owl monkeys were trained to sustain a hand grasp with 80 gms of force and a 250 Hz vibration for 1.5 sec/reward. Monkeys performed several hundred repetitions/day. In time:1) a tremor developed in grasping/ releasing the handpiece; 2) the speed of task performance declined; 3) weakness in closing the hand emerged; 4) increased task errors were recorded; and 5) difficulties emerged in other hand retrieval tasks. Compared to the control side, the representation of the trained hand in Area 3b was substantially degraded: 1) the size of the cortical hand and digit representations were reduced from normal; 2) receptive fields were, on average, many times larger than normal; 3) in the majority of cortical penetrations, neurons had multiple receptive fields extended across entire fingers or multiple digits; 4) in contrast to normal, neurons at many sampled sites had pacinian-like responses that covered much or all of the glabrous hand surface; 5) many large receptive fields extended over large sectors of both the volar glabrous and dorsal hairy hand surfaces; 6) receptive fields overlapped with each other over very long cortical distances; and 7) digit representations were geographically disorganized. We hypothesize that profound, experience-driven change in the quality of differentiated sensory feedback must contribute to the loss of movement control that marks the emergent focal dystonia. Human and monkey studies are now being directed toward determining how sensory learning could be implemented to re-differentiate cortical representational topographies, and restore normal motor control.
[Research supported by NIH Grant NS-10414, HRI and UCSF REAC].

692.4

DEPLETION OF BRAIN SEROTONIN DOES NOT IMPAIR PLASTICITY OF FUNCTIONAL REPRESENTATION OF VIBRISSAE IN RAT BARREL CORTEX. M.Kossut*, K. Turlejski, R.Djavadian Dept. of Neurophysiology, Nencki Institute, Warsaw, Poland.

Serotonin influences development of somatosensory cortex in rat (Bennet-Clarke et al., 1994) and the blockade of 5HT receptors impairs developmental plasticity of the visual cortex (Gu and Singer, 1991). We examined the effects of 5HT depletion upon plasticity of cortical representation of a row of vibrissae (visualized with 2DG) evoked by neonatal lesions of vibrissal follicles in hooded rats. On the day of birth pups were pretreated with desipramine HCl and one hour later received injection of 5,7 DHT. In one group unilateral vibrissectomy sparing row C was also performed. Serotonin immunostaining at the age of six weeks revealed that in rats injected with 5,7 DHT 70-90% of serotonergic fibers in the cortex were missing. 2-deoxyglucose (2DG) mapping of the row C representation was done on remaining rats. The vibrissae were clipped on both sides with the exception of row C, the rats were injected with C^{14} 2DG and the rows C on both sides were stimulated. Autoradiograms of brain sections cut tangentially to the barrel field were processed with an image analyzer and the sections were Nissl stained. Measurements of the size of barrels on the intact side showed that their linear dimensions in the 5HT depleted rats were 5-10% smaller than in untreated littermates. More than two-fold increase of the 2DG labeled cortical representation of row C was found in both control and 5HT depleted rats. Thus neonatal vibrissectomy resulted in very large increase of cortical representation of the spared row C despite destruction of serotonergic axons.
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692.5

OPTICAL IMAGING OF HAND REPRESENTATION IN PRIMATE AREA S1. D. Shoham*, D. Glaser and A. Grinvald. The Weizmann Inst. of Science, Rehovot 76100, Israel.

High resolution maps of cortical function organization *in-vivo* has both scientific and clinical importance. Optical imaging of intrinsic signals is particularly suited for this purpose. We are reporting here the first application of this method to the study of the primate somatosensory cortex.

Using mechanical tactile stimulation, we mapped the somatotopic representation of the hand in Macaque monkey area S1. The stimuli consisted of air-pressure pulses applied to various locations on the hand simultaneously. The skin was displaced either by using small flexible transducing membranes, or by fine, directed jets of air. We explored the effects of stimuli with different temporal properties, varying the number of pulses, pulse duration and inter-pulse intervals. In addition we looked at the effect of changing the air-pressure.

Using this stimulation method, we were able to see the cortical representation of each individual finger. In the maps we obtained, stimulation of each finger activated a narrow band running across the post-central gyrus. As expected, these stripes were ordered continuously along the gyrus. We also mapped responses to stimulation of the skin of the glabrous pads and of the forearm. The optical maps were confirmed by extensive multi and single unit recordings. All maps remained very stable over many hours of recording. Thus, it should be possible to use this method to reliably explore plasticity of the digit representation.

By optimizing the stimulus parameters, we were able to obtain the functional maps extremely rapidly. Averaging the responses to a very small number (~4) of stimulations was sufficient to produce clear maps. Thus, maps of the five digits could be obtained in as little as five minutes. This rapidity allowed us to apply this method for mapping functional borders in humans undergoing neurosurgery. In a hospital study we managed to localize the central sulcus and map the border of the hand representation in the somatosensory cortex. [Supported in part by the Israel Science Foundation administered by Israel Academy of Sciences and Humanities]

692.6

MINIMAL EXTENT OF VENTROPOSTERIOR LATERAL NUCLEUS OF THALAMUS CAPABLE OF MAINTAINING A SOMATOTOPIC MAP IN AREA 3b. P.R. Manger, T.M. Woods, E. Rausell* & E.G. Jones. Dept. Anatomy and Neurobiology, Univ. of California Irvine, Irvine, CA 92717

The inputs to the VPL thalamus from the dorsal column nuclei, and the projections from the thalamus to the somatosensory cortex, especially area 3b, show a high degree of somatotopic precision. Individual cells in VPL, however, show considerable divergence and overlap in their cortical projections (Rausell & Jones, J. Neurosci.: in press). We asked to what extent can this divergence maintain a map in area 3b in the face of diminishing numbers of VPL cells. Portions of VPL were electrolytically lesioned following physiological identification, and after two weeks, the body representation in area 3b was electrophysiologically mapped. The lesions involved up to 80% of VPL. Behavioral deficits included lack of appreciation of light tactile and proprioceptive stimuli, mainly in the contralateral hand. Reconstructions of the multiunit recorded cortical maps made after two weeks showed no apparent change in the somatotopic map of area 3b, even after the most extensive of the VPL lesions. Other cortical areas were not investigated. The preservation of even a few rod like bundles of cells of similar somatotopy within VPL may account for the maintained cortical map. If a small portion of the rod remains, the divergent connections may be sufficient to maintain the cortical map. However, the observed behavioral deficits suggest that the lesioned monkeys had problems with perception of stimuli. Further to this, the multiple inputs into area 3b, from other thalamic nuclei, callosal connections, and pain pathways, may also contribute to the maintenance of the cortical map despite the lack of a primary input.

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692.7

LOCAL CHANGES IN GENE EXPRESSION FOR GAD, $GABA_A$ RECEPTOR SUBUNITS AND CAM II KINASE IN SOMATOSENSORY CORTEX IN MONKEYS WITH VENTROBASAL THALAMIC LESIONS. T.M. Woods*, P.R. Manger, E. Rausell & E.G. Jones. Dept. Anatomy and Neurobiology, Univ. California Irvine, Irvine, CA 92717.

The ventrobasal complex of the monkey thalamus (VB) sends axons to ipsilateral postcentral gyrus in an ordered fashion. Recent evidence indicates that these thalamocortical projections exhibit substantial divergence and overlap (Rausell and Jones, J. Neurosci.: in press). This study addresses the issue of the extent to which this divergence can maintain a normal pattern of gene expression for molecules involved in plasticity of area 3b maps in the presence of reduced VB input. Lesions of varying extent were made in parts of the VB representation defined by multiunit mapping. After one or two weeks, the thalamus and somatosensory cortex were sectioned and hybridized with radioactive cRNA probes for glutamic acid decarboxylase (GAD), calcium/calmodulin dependent protein kinase α (CAMIIKα) and $\alpha 1$, $\beta 2$ and $\gamma 2$ subunits of the $GABA_A$ receptor subunit mRNAs, or stained immunocytochemically for GABA, GABA receptor subunits, CAMIIKα and parvalbumin. In general, the presence of a remaining portion of a VB representation was sufficient to maintain a normal pattern of gene expression, local changes only occurring in cortex with the largest lesions. Divergent connections, therefore, maintain sufficient activity to support a normal balance of inhibition and excitation in cortex.

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692.8

A ROLE FOR ASTROCYTES IN CORTICAL REORGANIZATIONAL PLASTICITY? F. Conti*, A. Minelli, and T.P. Pons°. Inst. Hum. Physiol., Univ of Ancona, I-60131 Ancona, Italy, and °Dept of Neurosurg., Bowman Gray School of Medicine, Winston-Salem, NC 27157, USA.

What triggers the mechanisms that determine cortical reorganization is poorly understood. Since the expression of transmitters is regulated by activity, it is possible that changes in transmitter levels could be such a triggering event. In this study, antibodies to glutamate (Glu) were used to study the effects of reduced afferent input on cortical excitatory neurons in adult monkeys subjected to somatosensory deprivation by cutting the three nerves to the hand.

In the cervical SC, DCN, VP thalamus and first somatic sensory cortex, Glu-immunoreactivity (Glu-ir) was similar to that described in normal animals; regions with reduced or absent Glu-ir were never observed and no appreciable differences were noted between the lesioned and normal side. No changes were observed in sections processed for CO or in thionin-stained sections. In the insulo-parietal operculum, sections contralateral to the nerve cut showed that most cortical fields had a normal pattern of Glu-ir (pattern *a*), some exhibited a reduction (pattern *b*), and that in the central portion of the upper bank of the central sulcus, which corresponds to the hand representation of the second somatic sensory cortex (SII), Glu-ir had virtually disappeared (pattern *c*). Adjacent sections processed for CO or stained with thionin showed that in the regions corresponding to those characterized by pattern *c*, CO slightly decreased and astrocytic proliferation was observed. Cortical regions characterized by pattern *c* also revealed that in the hand representation of SII, small-sized, intensely stained cells with processes radiating in all directions, which proved to be astrocytes, displayed Glu-ir.

As in previous studies, our findings indicate that Glu-ir is regulated by afferent activity. Additionally, changes in astrocytic Glu levels in the reorganizing cortex suggest a role for neuron-astrocyte/astrocyte-neuron signaling in the reorganization process. Such regulation of Glu levels in astrocytes and neurons may act as trigger for the biochemical changes underlying the structural metamorphosis occuring during a slow phase of reorganizational plasticity in the cerebral cortex.

692.9

LACK OF EXPRESSION OF $GABA_A$ RECEPTOR SUBUNIT mRNAs IN RETICULAR NUCLEUS OF THALAMUS. M. M. Huntsman* and E. G. Jones. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

The major fast inhibitory neurotransmitter receptor in the central nervous system, the $GABA_A$ receptor, contains binding sites for multiple ligands. The diversity of the receptor is dependent upon combinations of multiple subunits that make up a given stoichiometry that is reflected in the affinities displayed by each ligand. The receptor is a pentameric array formed potentially from at least 15 subunit polypeptides derived from individual genes with varying degrees of sequence identity organized into classes (e.g., α, β, γ, δ, ρ) and into class variants (e.g., α_1, α_2, α_3, α_4, α_5, α_6). Transfection of the α genes into cell lines or oocytes yields varying degrees of affinities for benzodiazepines in which strong affinity binding characterizes $\alpha 1$ and lower affinity binding $\alpha 2$-$\alpha 6$. cDNAs for the α_1, α_2, α_3, α_4, α_5, β_1, β_2, β_3, γ_1 and γ_2 subunits were subcloned from monkey total RNA, amplified via the polymerase chain reaction and inserted into the pBS transcription vector for the localization of ^{33}P-labeled RNA transcripts in sections of the thalamus. In situ hybridization of transcripts encoding individual subunit genes yielded a wide array of expression patterns throughout the monkey thalamus in which certain transcripts are expressed in a nucleus specific manner. In the reticular nucleus, most transcripts including α_1 and β_2, typically expressed at high levels in other nuclei, were not expressed although reticular neurons possess bicuculline sensitive $GABA_A$ receptors. Only the γ_2 transcripts showed detectable hybridization in the reticular nucleus. The presence of γ_2 subunit mRNAs without α subunits is interesting in light of its involvement in affinity enhancement for benzodiazepines and suggests the existence of novel subunits in this nucleus. Supported by NIH grant number NS21377.

693.1

SPATIAL ATTENTION PROTECTS MACAQUE V2 AND V4 CELLS FROM THE INFLUENCE OF NON-ATTENDED STIMULI.

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Last year we reported that spatial attention isolates cells in macaque area V2 from the influence of nearby, non-attended stimuli. In passive viewing, the response to a single flashing bar within the receptive field is often suppressed by a second, nearby flashing bar. However, when attention is directed to the location of the first stimulus, the suppression caused by the second stimulus is filtered out, and the cell responds as though the second stimulus were absent.

Our present results show that a related attentional process is at work in macaque area V4. As in V2, we found that the response to a single flashing bar within the receptive field is typically suppressed by the presence of a nearby, synchronous flashing bar. As in V2, this suppression is filtered out when attention is directed to the first flashing bar.

We have also recorded V2 responses when the flashing bar is presented against a variety of large bit-mapped "real world" images. The real world images typically activated the cells in a sustained fashion, but also suppressed responses to the bar. Attending to the flashing bar filtered out some, but not all of the sensory interactions resulting from these more complex stimuli.

693.2

TWO-DIMENSIONAL MAPS OF SPATIAL ATTENTION EFFECTS IN MACAQUE AREA V4.

C.E. Connor*, D.C. Preddie, J.L. Gallant and D.C. VanEssen. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

We have shown previously that responses in macaque area V4 depend on both the distance and direction of attention relative to the stimulus in the receptive field (Connor et al., *Soc. Neurosci. Abstr.* **19**: 974 (1993); Connor et al., *Soc. Neurosci. Abstr.* **20**: 1054 (1994)). Responses are generally stronger for stimuli near the attentional focus, and a majority of cells are tuned for direction. The optimal direction of attention (e.g., left, right, above, or below the stimulus in the receptive field) varies between cells. We designed a new experiment to map these directional attention effects in greater detail.

The monkey fixated a spot on a video monitor while a set of 12 ring-shaped stimuli was presented in a square array surrounding the classical receptive field of the cell under study. On a given trial the animal was required to attend continuously to just one of these rings and signal when it changed shape. Meanwhile the cell's response was tested by flashing 3 bars of optimal orientation, width and color spanning the central 50% of the classical receptive field. A majority of the cells tested in area V4 showed a significant effect of attention position in this paradigm. Spatial tuning for attention position was typically broad, with most cells showing greater than half maximal responses across at least a quadrant of the mapped region. The results provide further evidence that area V4 processes information about the positional relationship between the stimulus driving the cell and the current focus of attention.

693.3

TIME COURSE OF ATTENTIONAL EFFECTS IN MACAQUE AREA V4.

D.C. Preddie, C.E. Connor, J.L. Gallant* and D.C. Van Essen. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Recent experiments have shown that attentional effects on single cells in macaque area V4 include a shift in response strength towards the locus of attention and a change in overall response strength depending on the direction of attention (Connor et al., *Soc. Neurosci. Abstr.* **19**: 974 (1993); Connor et al., *Soc. Neurosci. Abstr.* **20**: 1054 (1994)). We designed a behavioral paradigm to assess the time course of these effects when attention is suddenly shifted. A macaque monkey was trained to attend to one stimulus (a ring) as part of a shape discrimination task, while a second, unattended stimulus (a bar) was used to map neural response profiles. Each trial began with the simultaneous onset of the fixation point, a probe (bar) stimulus in one of five positions within the classical receptive field (CRF) and a field of rings positioned outside the CRF. After a 1 sec delay the attended ring was cued via a brief (100 msec) blink at one of four positions surrounding the CRF. The animal was required to continuously monitor the attended ring and to release a response lever when, after a variable time interval, the ring became C-shaped.

Of 27 cells recorded thus far in one rhesus monkey, 10 showed a significant ($p < 0.02$) shift effect and 10 showed a significant directional effect. To determine the temporal profile of these effects, we analyzed the average responses in each group of cells. The shift effect began to develop at around 50-100 msec and peaked at about 200 msec following the attentional cue. In contrast, the directional effect developed more slowly, starting at about 200 msec and reaching a plateau 400-500 msec after the cue. These results suggest that the neural events underlying spatial shifts of attention occur rapidly, while information about the positional relationship between stimuli and attention evolves more slowly.

693.4

NEURAL CORRELATES OF TARGET DETECTION DURING VISUAL SEARCH IN AREA MT.

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Focal attention has been shown to play a central role in perception of complex objects. Visual search is a psychophysical paradigm traditionally used to explore the dynamics and mechanisms of focal visual attention. Using this paradigm we have previously shown that both humans and rhesus monkeys exhibit serial search for targets defined by a conjunction of color and direction of motion, i.e., search times depend linearly on the number of distractors in a search array (Buracas and Albright, 1994, *Soc.Neurosci.Abs*, **20-1**, p.318). This linear dependency is commonly accepted as evidence for dynamical and voluntary allocation of focal attention.

Using an identical search paradigm, we have now explored neurophysiological correlates of focal attention in rhesus monkeys. Specifically, we have recorded responses of directionally selective neurons in area MT of a monkey engaged in a visual search task. The search target was formed by a unique conjunction of color (red or green) and direction of motion (preferred or null direction for neuron under study) of a texture inside a circular aperture. The target for each trial was specified by a sample presented prior to the search array. The latter was composed of eight apertures defined by all possible combinations of selected color and direction. The monkey was required to saccade to the target upon detection, or to maintain central fixation on target-absent trials. Approximately 40% of MT neurons exhibited significant response modulation when the search target was positioned within the receptive field. This modulation was expressed as an enhancement (~30% of the predicted baseline) of the neuronal response prior (90-200 ms) to a saccade to the target. The time course of the neuronal response modulation thus parallels behavioral target detection. The sign of the modulation is consistent with the process of target selection and may reflect the engagement of an attentional mechanism.

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693.5

THE INFLUENCE OF ATTENTION AND INTENDED EYE MOVEMENTS ON THE VISUAL EXCITABILITY OF NEURONS IN THE LATERAL INTRAPARIETAL AREA OF MACAQUE MONKEYS

J-R. Duhamel*, S. Ben Hamed, F. Bremmer and W. Graf C.N.R.S.-Collège de France, F-75292 Paris Cedex 06, France.

Visual activity of neurons in the monkey lateral intraparietal area (LIP) is influenced by the direction of attention and by eye movements. To understand better how these contribute to spatial processing, we investigated the tuning of LIP neurons to visual stimulation. Single cell responses were recorded extracellularly in one rhesus monkey (*Macaca mulatta*) under three different conditions: (1) during a fixation task in which the monkey attended to a small foveal stimulus and released a hand-held bar when the color of this stimulus changed at an unpredictable time, (2) during the delay period of a memory-guided saccade task (e.g. between the presentation of a brief peripheral target and the beginning of the saccadic eye movement), and (3) during spontaneous gaze without fixation or saccade targets.

Under all conditions, the visual excitability of the recorded neurons was assessed by presenting randomized sequences of test flashes (100 msec) at a rapid rate (100-250 msec interval) during periods of stable eye position. This enabled the sampling of a large number of locations within a 80° by 80° portion of the visual field, and the subsequent generation of surface plots for a given cell's receptive field (RF). Many LIP neurons displayed graded responses with a maximum at the center of the RF, decreasing toward the periphery. In some cells, the area of excitability was quite restricted and had sharp borders. In other cells, the RF had a complex surface with both excitatory and inhibitory zones. The state and direction of visual attention had an effect on overall responsiveness but also influenced different characteristics of the RF configuration such as sharpness of spatial tuning, and center of gravity. This indicates that the encoding of visual space by parietal visual neurons is strongly linked to the behavioral context.

693.6

ATTENTIONAL MODULATION OF DIRECTION-SELECTIVE RESPONSES IN THE SUPERIOR TEMPORAL SULCUS OF THE MACAQUE MONKEY.

S. Treue* and J.H.R. Maunsell Baylor College of Medicine, Houston, TX

We recorded from over 50 direction-selective cells in the superior temporal sulcus of a macaque monkey performing a reaction-time task to investigate the role of attention in the processing of motion signals. The monkey was trained to attend a moving spot (the target) in the presence of one or more other moving spots (distractors) while maintaining fixation on a stationary fixation cross. In the first experiment one spot moved through the receptive field (RF) in the preferred direction while another spot moved outside the RF. Even though retinal stimulation was identical in both trial types a large majority of the cells (~85%) responded more strongly when the animal was instructed to attend to the stimulus within the RF, compared to trials on which he was attending the stimulus outside the RF (median increase 30%). In the second experiment both spots moved through the RF, but in opposite directions (preferred and anti-preferred direction of the neuron). Allmost all cells (~90%) responded more strongly when the monkey was instructed to attend to the stimulus moving in the preferred direction, compared to trials in which he was attending the spot moving in the anti-preferred direction (median increase 150%). This result cannot be accounted for by shrinking receptive fields since it held true even when the two dots passed by each other very closely.

Our results demonstrate that while moving attention away from the receptive field will reduce a neuron's response to its preferred stimulus a much stronger effect can be observed when attention is switched between two stimuli that both fall within the receptive field. In that case the response of the neuron is strongly biased by the direction of motion of the attended stimulus even when the unattended stimulus is a more potent sensory stimulus. These findings are amongst the strongest extraretinal modulations of neural signals found in visual cortex to date and suggest an important role for attention in selectively enhancing neural signals related to stimuli of interest.

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693.7

DISTINGUISHING CORTICAL AREAS THAT ARE SENSITIVE TO TASK AND STIMULUS VARIABLES WITH FMRI E.A. DeYoe*, P.W. Schmit, & J. Neitz Dept. Cell. Bio., Med. Col. Wisconsin, Milwaukee, WI (Email: edeyoe@its.mcw.edu)

Purpose. Functional MRI was used to study the effects of a) stimulus presentation rate and b) task requirements on cortical activation induced by a visual stimulus. **Methods**. During gradient-recalled, echo-planar FMRI, 3 subjects viewed a flickering checkered annulus under two task conditions: 1) passive viewing and 2) active target discrimination. Single stimulus presentations lasted 0.5 sec, and were repeated at 4 different rates (from 0.2 to 2 per second) in blocks (trials) of 24 seconds each. For each voxel, FMRI response amplitudes were extracted for the different presentation rates and curve-fit by linear regression. Histograms of regression line slope revealed two asymmetric peaks. Voxels whose response increased with presentation rate were classified as rate dependent while those with constant response were classified as rate independent. **Results**. Voxels in V1 and several extrastriate visual areas showed strong stimulus rate dependence in both task conditions. However during passive viewing, stimuli had to be presented nearly twice as fast as during active viewing to achieve the same response magnitude in V1. Such differences were even larger for extrastriate visual areas. In frontal cortex, results were distinctly different. The response was rate independent during active viewing, while under passive viewing there was little or no response. **Conclusions**. Stimulus rate-dependence may provide a method for distinguishing areas of the brain serving task-related functions such as attention from those areas primarily concerned with processing of visual information. Supported by NIH grants EY10244, and Core Grant EY01931.

693.8

FMRI REVEALS FEATURE-SPECIFIC ATTENTIONAL MODULATION OF AREA MT, V3/V3a AND PARIETAL VISUAL AREAS. Michael S. Beauchamp* and Edgar A. DeYoe Dept. Cell Biology & Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226 (mbeaucha@mcw.edu)

Visual stimuli consisting of motion-defined figures were used to study human visual areas important for motion perception. Attention to different stimulus features served as a powerful modulator of activation. In the attention-to-motion condition, subjects made a discrimination based on slightly different point speeds in the left and right halves of the figure. In the attention-to-luminance condition, subjects responded to slight luminance increments and decrements of the fixation point. The identical visual stimulus was presented in both conditions, consisting of a phasically displayed (5 on/off cycles) annulus defined by the coherent motion of its component points on a continuously displayed background of equally-distributed incoherently moving points with a varying luminance fixation point. Visual stimuli were generated with a PC-based graphics system and projected through a Maxwellian-view optical system, allowing a 60 degree field-of view, while whole-brain echo planar images were collected with a custom head coil in a 1.5 T scanner with voxel size 56 25 mm^3 (3.75x3.75x4 mm.)

When subjects performed the attention-to-motion task, the higher visual areas specialized for motion processing which presumably make explicit the presence of the motion-defined annulus were strongly activated. Putative human visual area MT (hMT) showed consistent activation (volume of activation 16 ± 6 voxels, mean ± SD, n=4) as did visual area V3 (12 ± 4 voxels.) Several parietal areas were also activated in the attention-to-motion condition (84 ± 23 voxels.) When subjects viewed the identical stimulus in the attention-to-luminance condition, the area of activation was sharply reduced. hMT fell 98% to 0.3 ± 0.5 voxels, V3 fell 94% to 0.8 ± 1.5 voxels and parietal areas were reduced by 93% to 6 ± 13 voxels. Primary visual cortex responded similarly to the random points whether they were moving coherently or incoherently, resulting in relatively little phasic response in either attentional condition (attention-to-motion: 8 ± 4 voxels, attention-to-luminance: 1 ± 2 voxels.) Supported by a HHMI Fellowship to MSB and NIH RO1-EY10244 to EAD.

693.9

COMBINED fMRI, EEG AND MEG IMAGING OF VISUAL ATTENTION

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The activation of human cortex was compared in four subjects using MEG, EEG and fMRI in visual selective attention experiments related to that of Corbetta et al. (J. Neurosci. 1991) . Event-related magnetic fields and electric potentials were compared to hemodynamic changes observed using functional MRI. The passive condition was to fixate on a cross and ignore the stimuli moving across the screen every 700 ms. In the color, orientation, and movement conditions, the subject saw the same stimuli, but the task was to attend to stimulus color, orientation, and movement direction, respectively. Movement-corrected fMRI data from each subject were registered and averaged in Talairach space. Multiple areas were differentially activated during the attended conditions, including extrastriate, parietal, dorsal lateral prefrontal and anterior cingulate cortices. Multiple and temporally overlapping sources were observed using MEG and EEG. In the passive condition, electromagnetic methods show activity in V1 and V2 areas, with latencies from 70 to 160 ms. Signal strength increases were observed across all modalities during the attended conditions, with attention to color giving the largest responses in this particular task. During the attend condition, fMRI, MEG and EEG suggest that the strength of these sources is modulated by attention.

693.10

REVERSIBLE VISUAL HEMINEGLECT IN THE CAT.

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Following unilateral damage of cortex at the temporo-occipito-parietal (TOP) junction, humans fail to notice and respond to stimuli that appear in visual space contralateral to the lesion. In cases of profound neglect, subjects neither detect nor orient head or eyes to stimuli appearing in the contralesional field. Similar, but less impressive, visual defects have been obtained following restricted unilateral lesions of TOP cortex in monkeys. We have identified a region at the TOP junction of the freely moving cat which, when cooled and inactivated unilaterally, results in a profound neglect of stimuli introduced into the contracooled hemifield. We also show that the severity of the defect matches that induced by unilateral cooling of the superior colliculus. The cortical region in the cat is located in the posterior one-half of the middle suprasylvian sulcus at the junction of areas believed to be equivalent to primate areas forming the V5 complex, and areas V4 and 7/39. The broad similarity of cooling induced- and lesion induced- neglect in cats, monkeys and humans suggests that there are largely equivalent circuits in all three species that likely include the superior colliculus. The link between TOP cortex and the orienting defect may be a useful fiduciary marker that will contribute to the identification of a number of extrastriate areas in the human brain, for which there are, as yet, very few markers. Supported by NS 32137.

693.11

BASIS FUNCTIONS AND HEMINEGLECT A. Pouget*[1] and Sejnowski, T.J.[2]. [1]Brain Research Institute, UCLA and [2]Salk Institute, San Diego.

Cortical lesions in the parietal lobe of human produce a neurological syndrome called hemineglect, in which patients tend to ignore sensory stimuli in the hemispace contralateral to the lesion. Several studies attempted to determine the spatial frame of reference affected in visual neglect and found that it can be retinotopic, head-centered, body-centered, environment-centered, and in some cases, object-centered, all in the same patient.

We recently developed a theory of spatial representations in the parietal cortex based on the responses of single parietal neurons and the computational nature of sensorimotor transformations. The model assumes that parietal neurons encode their sensory inputs with basis functions (BF), a type of receptive fields that can be used to generate nonlinear motor commands. One of the important property of BF map is that the position of an object can be represented in several frames of reference simultaneously. We simulated a lesions of BF maps and found that, as observed in hemineglect, the resulting deficits affect multiple frames of reference, including object-centered. This model can also account for recovery from neglect that occurs over weeks and transiently after caloric vestibular stimulation. The model demonstrates that neglect can be explained without assuming the existence of explicit representations of cartesian space or object-centered representations. It relies instead on basis function maps biased for the contralateral side of space. This is, to our knowledge, the first account of hemineglect at the single cell level.

694.1

CENTRAL SENSITIZATION IN CHRONIC CENTRAL PAIN AFTER SPINAL CORD HEMISECTION. C.E. Hulsebosch*, M.D. Christensen, Y.B. Peng and W.D. Willis. Dept. of Anat. and Neurosci. and Marine Biomed. Inst., Univ. Texas Med. Br., Galveston, TX 77555-1069.

A model of chronic central pain after spinal cord injury (SCI) was used to document the temporal development of mechanical allodynia using validated models of nociceptive behavior. The same animals tested behaviorally were used to determine if dorsal horn neurons were more excitable to peripheral stimulation as evidenced by increased responses to brush, press, pinch, von Frey hair stimulation following spinal cord hemisection at T13. Electrophysiological recordings of wide dynamic range (WDR) neurons in the dorsal horn from L3-L5 were made both contralateral (n=25) and ipsilateral (n=25) to the hemisection. These data were compared to control values (n=32). The responses were recorded as spikes per second, before, during and after the application of these natural stimuli. The responses to brush, pinch and von Frey stimulation at 9.41mN were increased in the hemisected rats bilaterally and these increases were statistically significant compared to control values ($p<0.05$). These data support the development of central sensitization of WDR neurons and provide a mechanism for the observed behavioral changes in the development of mechanical allodynia after SCI. (Supported by NIH grants NS 11255, NS 09743 and the Kent Waldrep Foundation)

694.2

PERSISTENT, CHEMICAL NOCICEPTION IN THE RAT REQUIRES ONGOING PERIPHERAL NERVE INPUT. B.K. Taylor*, M.A. Peterson and A.I. Basbaum. Depts. Anatomy, Physiology, and W.M. Keck Foundation Center for Integrative Neurosciences, UCSF, San Francisco, CA.

Hindpaw injection of formalin produces acute (Phase 1) and persistent (Phase 2) nociceptive behaviors in the rat. This model has provided critical evidence supporting a contribution of central sensitization (hyperexcitability of spinal neurons) to the expression of persistent pain. Here, we evaluated the contribution of ongoing peripheral nerve inputs to Phase 2 pain responses. In addition to pain behavior (flinching), we measured formalin-evoked increases in arterial pressure and heart rate via an indwelling arterial catheter. All three measures displayed both phases of the formalin response. The arterial pressure response was highly correlated with behavior, and was dependent on formalin concentration (0.625 - 5.0%), indicating that it was largely driven by nociceptive input. Animals obtained from Charles River exhibited slightly, but significantly, larger Phase 2 flinching and heart rate responses compared to those from Bantin & Kingman. Lightly-anesthetized (0.7% halothane) rats exhibited robust increases in blood pressure, indicating that cardiovascular responses did not merely reflect somatomotor-cardiovascular coupling. We next evaluated the contribution of ongoing peripheral nerve activity to the expression of the Phase 2 pressor, tachycardia, and flinching responses. We locally-anesthetized the ipsilateral or contralateral (control) paw with a hydrophillic lidocaine derivative, QX-314 (2%) after Phase 1, but before Phase 2 began. QX-314 blocked Phase 2 pressor, tachycardia and behavioral responses only when injected into the paw that received formalin (2.5% or 10.0%). We conclude that ongoing activity from peripheral nerves is required for the persistent pain evoked by formalin. Supported by NS21445, DA 08377 and NS 07265.

694.3

RELATIONSHIP OF OPIOID RECEPTORS TO NEURONS IN NUCLEUS RAPHE MAGNUS (NRM) AND THE PERIAQUEDUCTAL GRAY MATTER (PAG) IN RATS AND MONKEY. M Wessendorf*, M Riedl, CN Honda, S Schnell, R Elde, and U Arvidsson. Dept Cell Biology and Neuroanatomy, Univ Minnesota, Minneapolis, MN 55455

Opiate analgesics may act in part by activation of neurons in PAG, which in turn activate spinally projecting neurons in brain stem regions such as NRM. In the present study, the relationship between delta and mu opioid receptors and PAG and NRM neurons was examined. Rats and one Rhesus macaque were deeply anesthetized and sacrificed by perfusion with 4% formaldehyde. Tissue sections were stained using antisera against N-terminal portions of the delta receptor and against the C-terminus of the mu-receptor. Both PAG and NRM in both rats and monkey were stained by antisera against both mu and delta receptors. However, in both species, delta antisera generally labeled fibers whereas the mu antiserum labeled cells. Spinally projecting NRM cells were retrogradely labeled in 3 rats and NRM sections were doubly stained for serotonin and delta receptors. When sections were examined by confocal microscopy, it was found that retrogradely labeled serotonin-stained cells were frequently apposed by varicosities positive for delta receptors.

These findings suggest immunocytochemical staining can localize opioid receptors in NRM and PAG of both rats and primate. In addition, they suggest that delta opioid receptors modulate the activity of afferents to raphe-spinal serotonergic neurons.

These studies were supported by DA 05466, DA 06299, and by the Minnesota Medical Foundation.

694.4

THE EFFECTS OF STIMULATION OF THE MEDIAL PREOPTIC NUCLEUS (MPO) ON PAG NEURONS AND BLOOD PRESSURE. M. M. Behbehani* T.M. Da Costa Gomez, M. Ennis and M.T.Shipley Dept. of Mol. and Cell. Physiology, U. of Cincinnati Cincinnati, OH 45267-0576 and Dept. of Anatomy, U. of Maryland at Baltimore, Baltimore, MD 21201.

The MPO is involved in a variety of functions including lordosis and pain inhibition. This area has an extensive projection to the periaqueductal gray (PAG). In this study we examined the effect of electrical and chemical stimulation of the MPO on the baseline activity of PAG neurons and examined the role of glutamic acid in this pathway. We recorded from 88 PAG neurons of 22 chloral hydrate anesthetized male rats and continuously monitored the arterial blood pressure. Electrical stimulation (50-200 uA, 100 Hz, 10 sec.) excited 25 cells, inhibited 11 cells, and had no effect on 28 cells. Chemical stimulation (50-100 nl, 10 mM dl-homocysteic acid) excited 8 cells, inhibited 8 cells, and had no effect on 34 cells. Microinjection of the glutamic acid antagonist kynurenic acid in the PAG blocked the excitatory response of 10/20 cells and the inhibitory response of 3/10 cells to MPO stimulation. The cells that were excited had a mean firing rate of 4.89 ± 1.18 Hz, inhibited cells had a mean firing rate of 8.45 ± 1.41 Hz, and unresponsive cells had a mean firing rate of 8.75 ± 1.33 Hz. The majority of the cells that were excited by MPO stimulation were also excited by noxious pinching of the tail. There was no difference between the response of cells that were inhibited by MPO stimulation and their response to noxious stimulation. In the majority of animals (18/22), MPO stimulation produced a depressor effect that ranged between 5 and 30 mmHg with a mean of 15.9 ± 2.3 mmHg. A pressor effect was observed in 1/22 animals with an increase of 19 mmHg. It is concluded that MPO stimulation has a significant effect on the activities of PAG neurons, which is possibly glutamic acid mediated, and produces a decrease in the blood pressure. Supported by NIH grant NS20643.

694.5

MASSIVE EXPRESSION OF C-FOS PROTEIN IN SUPERFICIAL DORSAL HORN IS NOT FOLLOWED BY LONG-TERM CHANGES IN NOCICEPTIVE THRESHOLDS. H. Beck *. J. Sandkühler, X.G. Liu and A.-C. Treier. II.Physiologisches Institut, Univ. Heidelberg, 69120 Heidelberg, Germany.

We have tested whether the massive expression of immediate-early genes including c-*fos* is a sufficient condition for the induction of thermal or mechanical hyperalgesia in awake-drug free adult rats. A minimally invasive intrathecal (i.t.) stimulation and injection technique was used for electrical stimulation of dorsal roots (0.5 ms pulses, 15 V, 3 Hz for 15 min) or for i.t injection of 25 nmoles NMDA under brief ether anesthesia.

Both i.t. stimulations produced a massive expression of c-Fos protein mainly in laminae I-II from the sacral to lower thoracic spinal cord (n=10) as compared to ten sham-treated animals. Nociceptive cutaneous mechanical (v.Frey-hairs) and thermal (hot-plate and tail-flick tests) withdrawl thresholds were determined once daily for two weeks before and for two weeks after conditioning i.t. stimulation or sham treatment (n=10). Nociceptive thresholds were not different before and after conditioning stimulation or between stimulated and sham-treated animals.

Thus, even the massive expression of c-Fos in the superficial dorsal horn is not a reliable indicator of long-term changes in spinal nociception. Supported by the DFG.

694.6

CAUDATE NUCLEUS ACTIVATION IN RESPONSE TO INTENSE PAINFUL STIMULATION IS OBSERVED USING FUNCTIONAL MAGNETIC RESONANCE IMAGING

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The basal ganglia are believed to play a role in nociception and pain. Electrophysiological studies in cats have shown caudate nucleus neuron activation in response to high-intensity electrical stimulation of the inferior dental nerve sufficient to elicit the jaw opening reflex (Lidsky et al., 1979, *Brain Research Bulletin* 4, 9-14). In this study we examined caudate activation in humans during intense painful stimulation.

In 5 male volunteers 2 levels of painful electrical stimulation were assessed, as well as a non-painful brush stimulus. During the 4.5 minute scanning sessions, 32 second periods of stimulation were alternated with 32 second periods of rest. The first stimulus level, which was painful yet tolerable for the entire scanning session, was selected by the subject. The mean intensity of this 2 Hz stimulus was 14.8 mA. The second level was a 100 Hz 50 mA stimulus. This stimulus was administered after subjects were anesthetized with 0.7% Isoflurane and paralyzed with Vecuronium 0.15 mg/kg. Anesthetized subjects were also scanned during lower level electrical and brush stimulation.

Functional images were obtained using a 1.5T GE scanner with a birdcage RF coil designed for whole-volume brain imaging. A gradient-echo EPI imaging sequence was used with a TE of 40ms and a TR of 2 sec. All time course data was correlated with a boxcar function and a correlation threshold of 0.35.

Caudate activation was not seen in the anesthetized and unanesthetized scans of lower level electrical or brush stimulation. However, during the intense electrical stimulation of anesthetized subjects bilateral caudate activation was seen in 4 of the 5 subjects. Consistent activation of primary and secondary somatosensory cortices was also observed.

694.7

EFFECTS OF ANTICIPATION OF VISCERAL PAIN ON THALAMIC ACTIVITY IN IRRITABLE BOWEL SYNDROME PATIENTS AND NORMAL SUBJECTS. DHS Silverman, H Ennes, J Munakata, CK Hoh, M Mandelkern, J Markham*, ME Phelps, W Blahd, EA Mayer. Depts. of Medicine and Nuclear Medicine, UCLA Medical Center and West LA VA Medical Center, Los Angeles, CA, 90024.

Anticipatory anxiety is prominent in many patients with irritable bowel syndrome (IBS), as is lowered threshold to rectal pain. Anticipation of pain can modulate its processing and ultimate perception. In the current study, the relationship between anticipation of rectal pain and regional cerebral blood flow was evaluated in IBS patients and normal subjects (NIs) using $H_2{}^{15}O$ PET. A rectal balloon catheter was placed in each subject 30 min prior to imaging sessions. Following IV administration of 40 mCi $H_2{}^{15}O$, PET data were obtained in dynamic frames, and summed for 75 sec proceeding entry of tracer into the brain. Eyes were closed during all scans. A baseline scan was first obtained with the balloon deflated. Then, pressure pulses of low (20 mm Hg) and moderate (45 mm Hg) intensity were delivered by a programmable pump for 45 sec each, with interim balloon deflation (20 min). A taped message subsequently announced the imminence of a very intense pressure pulse. Heart rate was recorded before and after this message. A scan was performed following the announcement, while the balloon actually remained deflated. Relative activity was measured in various brain regions normalized to the activity level in the superior occipital cortex, a quiescent area that was unaffected by this protocol or IBS status. During anticipation, NIs showed a significant reduction in thalamic activity compared with their baseline level ($p<0.05$); in contrast, thalamic activity in IBS patients failed to change significantly and was higher than in normals. Heart rate changes during anticipation were strongly positively correlated with thalamic activity in both groups ($r = 0.98$). No significant differences were detected in the activity of other brain regions, including the anterior cingulate cortex, medial temporal lobes, temporal poles, or cerebellum. The aberrant response of the thalamus in IBS patients may reflect dysregulation of central processing of visceral pain information.

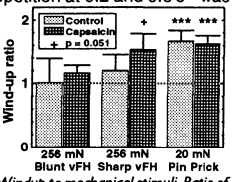
694.8

ENHANCED PAIN SENSITIVITY TO PUNCTATE STIMULI AND 'WIND-UP' OF PAIN SENSATION IN THE AREA OF SECONDARY HYPERALGESIA **W. Magerl, S. Wilk and R.-D. Treede (SPON: ENA*)** Inst. Physiol. Pathophysiol., Joh. Gutenberg Univ., D-55099 Mainz, F.R.G.

Skin injury triggers an enhanced pain sensitivity in surrounding uninjured skin (secondary hyperalgesia) due to heterotopic central sensitization. Repetitive stimulation of nociceptors at frequencies > 0.5 s^{-1} induces a homotopic response facilitation in dorsal horn neurons ('wind-up'), which is thought to contribute to central mechanisms of hyperalgesia. We therefore examined the presence and possible changes of perceptual wind-up in the area of secondary hyperalgesia.

Secondary hyperalgesia was induced by i.d. injection of capsaicin (40 µg). Sensitivity to mechanical stimuli using cotton balls, calibrated von Frey hairs (vFH) and pin pricks was tested at 15 mm distance from the injection site. Blunt vFHs (0.5 - 4100 mN) were used to determine S/R functions for pricking pain. Stimulus repetition at 0.2 and 0.6 s^{-1} was used to test for perceptual wind-up.

Capsaicin evoked strong burning pain and a leftward shift of S/R function (> factor 3) in the area of secondary hyperalgesia; noxious stimuli (pin pricks) were more painful and non-noxious mechanical stimuli eventually became painful. Though the pain ratings were increased in the area of secondary hyperalgesia, the extent of wind-up remained unchanged. Significant wind-up was only observed to pin prick repetition at the 0.6 s^{-1} frequency (see Fig.).



Windup to mechanical stimuli. Ratio of first vs. average of 5th - 8th pain ratings

We conclude, that the adjacent injection of capsaicin causes a pronounced enhancement of cutaneous pain sensitivity to punctate mechanical stimuli, but no equally pronounced change in wind-up to repetitive stimulation.

694.9

PAIN PERCEPTION OF NERVE CONDUCTION STUDIES: EFFECT OF STIMULUS DURATION AND INTENSITY. D.R. Del Toro*, A.F. Goldbaum, N.A. Johnson, T.A. Park. Department of Physical Medicine & Rehabilitation, Medical College of Wisconsin, Milwaukee, WI 53226.

Present electrophysiologic guidelines allow for significant variability in the stimulus parameters used for routine clinical nerve conduction studies (NCS), with little consideration given to the effect of these parameters on patient discomfort. Previous research suggests that some patients perceive these NCS as aversive or painful. This study was designed to examine the effect of stimulus duration and stimulus intensity on the perception of pain in individuals who have never undergone standard NCS. To study this effect, we performed a conventional median sensory antidromic conduction technique to the index finger of one hand in twenty asymptomatic subjects. Five different stimulus durations (50µsec, 100µsec, 200µsec, 500µsec, 1000µsec) were compared. Stimulus current (mA) was adjusted for each subject to 20% supramaximal at each stimulus duration. Immediately following each stimulation, all subjects rated the severity of pain perceived using a visual analog scale (VAS) ranging from no pain to the most intense pain possible. In addition, the State-Trait Anxiety Inventory was administered to each subject before and after testing. None of the stimuli were rated as more than mildly painful. Mean pain ratings for all stimulus durations were in the lower 40% of the VAS. Stimulus duration and pain rating were found to be inversely related. Shorter stimulus durations resulted in significantly higher pain ratings. There was no significant correlation between pre- or post-test anxiety scores and pain ratings. Results of this study may provide information which is useful in decreasing pain perception and anxiety associated with clinical NCS.

694.10

ROLE OF ENDOGENOUS CANNABINOIDS IN PAIN MODULATION J. Michael Walker*, William J. Martin, Andrea G. Hohman, Nicole M. Fortin, Dale G. Deutsch and Kang Tsou. Schrier Research Laboratory, Departments of Psychology and Neuroscience, Brown University, Providence, RI 02912, and Depts. of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY 11794.

For many years it has been known that the brain contains nonopiate modulators of pain sensitivity, because certain forms of stress- and electrical stimulation-produced analgesia are insensitive to naloxone. Little is known about the particular neural systems that mediate such effects. Recently, we have found evidence for the existence of a powerful neural system that modulates pain through a cannabinoid mechanism. Several lines of evidence indicate the existence of this endogenous cannabinoid pain modulatory system: 1) low doses of cannabinoids administered systemically, intracerebrally, or intrathecally produce profound analgesia; 2) cannabinoids inhibit noxious stimulus-evoked c-fos expression in spinal cord, and the firing of wide dynamic range neurons in spinal cord and thalamus; 3) increasing the synthesis of the endogenous cannabinoid anadamide by administration of the precursor ethanolamine produces cannabinoid receptor-mediated analgesia; 4) increasing the synaptic concentration of anadamide by administration of breakdown inhibitors produces profound cannabinoid receptor-mediated analgesia. Intracerebral microinjection studies suggest that these effects occur via actions in the dorsolateral periaqueductal gray, the dorsal raphe nucleus the rostral ventral medulla, the lateral posterior nucleus of the thalamus, the superior colliculus, and the nucleus submedius. These data demonstrate that endogenous cannabinoids play a role in the modulation of pain.

694.11

INHIBITION OF PLASMA PROTEIN EXTRAVASATION BY 5-HT_1 AGONISTS IN RODENT DURA MATER: PHARMACOLOGICAL CHARACTERIZATION USING A SELECTIVE ANTAGONIST AND 5-HT_{1B} KNOCKOUT MICE. C. Waeber, X.-J. Yu, N. Castanon, K. Scearce, R. Hen, J.E. Macor and M.A. Moskowitz*. Massachusetts General Hospital, Charlestown, MA 02129

5-$HT_{1B/1D}$ agonists have been shown previously to inhibit plasma protein extravasation in the dura mater of rodents (a model of migraine). However, the potency of some of these drugs in this paradigm does not correlate with their affinity at 5-$HT_{1B/1D}$ binding sites. To investigate the 5-HT receptor subtype mediating the effects of these agents, we have used 1) a specific 5-HT_{1D} antagonist in guinea-pigs and 2) knockout mice lacking 5-HT_{1B} receptors. Pentobarbital-anesthetized animals were injected with [^{125}I]BSA 5 min prior to unilateral electrical stimulation of the trigeminal ganglion. After intracardiac saline perfusion, the left and right dura were harvested and the ratio of extravasated [^{125}I]BSA (stimulated/unstimulated) measured. This ratio was close to 1.7 in vehicle-treated animals. Complete inhibition of extravasation (ratio close to 1.0) was achieved in both guinea-pigs and mice when sumatriptan (0.7µmol/kg), CP-122,288 (1 nmol/kg) and 5-CT (1 nmol/kg) were injected i.v. 10 min prior to trigeminal stimulation; the selective 5-HT_{1B} agonist CP-93,129 (1.4µmol/kg) was effective only in mice. In guinea-pigs, pretreatment with GR-127,935 (0.1 mg/kg, i.v., 20 min prior to agonists) reversed the effects of sumatriptan, but did not affect those of CP-122,288 and 5-CT. In 5-HT_{1B} knockout mice, CP-93,129 and sumatriptan were inactive, while CP-122,288 and 5-CT were as potent as in wild-type mice to inhibit extravasation. These results suggest that CP-93,129 (in mice) and sumatriptan act only via 5-$HT_{1B/1D}$ receptors to inhibit dural protein extravasation in rodents. In contrast CP-122,288 and 5-CT might act via a presently unknown receptor; the hypothesis is in agreement with the fact that their potency in this model is much higher than expected from their affinity at 5-$HT_{1B/1D}$ receptors.

694.12

ALPHA-2 ADRENERGIC AGONISTS PREVENT NMDA ANTAGONIST NEUROTOXICITY. N.B. Farber* and J.W. Olney. Dept. Of Psychiatry, Washington University, St. Louis, MO 63110.

Antagonists of the NMDA receptor are potentially useful neurotherapeutic agents for prevention of opiate tolerance, alleviation of neuropathic pain and prevention of neurodegeneration in stroke and brain trauma. However, the ability of these agents to reversibly injure or kill neurons in rodent brain and to produce psychotic "emergence reactions" in humans has raised concerns about their clinical use in humans. Over the past several years we have been studying the mechanism(s) underlying these adverse CNS side-effects and have proposed that they might represent different manifestations of the same toxic process. Here we report that the neurotoxicity induced in rat brain by the potent and specific NMDA antagonist, MK-801, is prevented by several α_2 adrenergic agonists in a dose dependent fashion. The dose of MK-801 used (0.5 mg/kg sc) causes a reversible neurotoxic reaction ("vacuole reaction") in specific cerebrocortical neurons in 100% of treated rats. The ED_{50}s (dose that reduced the mean number of vacuolated neurons by 50%) for the α_2 agonists tested were clonidine (0.044 mg/kg), guanabenz (0.21 mg/kg), lofexidine (0.38 mg/kg), p-iodoclonidine (1.3 mg/kg) and xylazine (2.4 mg/kg). If a common mechanism does underlie the neurotoxic and psychotomimetic effects of NMDA antagonists, then co-adminstration of an α_2 agonist with an NMDA antagonist might prevent the NMDA antagonist from inducing either of these adverse side effects. This would enable NMDA antagonists to be used more safely for alleviation of neuropathic pain (unresponsive to opiates but possibly responsive to α_2 agonists as well as NMDA antagonists) and to enable opiate analgesics to be used without addiction liability in the management of other forms of chronic pain. *Supported by DA07261, DA 05072, AG 11355, NARSAD* Established Invest Award, RSA MH 38894 (JWO) & Scottish Rite Benevolent Foundation's Schizophrenia Research Program (NBF).

695.1

CORTICAL DYNAMICS OF WORD RECOGNITION IN NORMAL AND DYSLEXIC SUBJECTS: A NEUROMAGNETIC STUDY. R. Salmelin*, E. Service, P. Kiesilä, and K. Uutela. Low Temp. Lab., Helsinki Univ. of Technology, 02150 Espoo, and Dept. of Psychology, Univ. of Helsinki, 00170 Helsinki, Finland.

We explored spatiotemporal cortical organization during word recognition in 7 normal subjects and in 5 subjects with a documented history of developmental dyslexia, employing a whole-head neuromagnetometer (Neuromag-122). All subjects were behaviourally tested for reading, naming, lexical decision, and mental rotation speed, and for digit span forwards and backwards.

Subjects were shown 7-8 letter words for 300 ms (centered, 4 deg visual angle), once every 3 s. Four categories alternated randomly: concrete and abstract Finnish words, pronounceable pseudowords without meaning, and unpronounceable, meaningless letter strings with the same amount of consonants and vowels as in the other classes. The subjects were instructed to say aloud the word 'kirahvi' (giraffe) whenever it appeared (2.5% probability).

Activation of primary visual areas was accompanied by lateral occipitoparietal signals within 200 ms after stimulus onset, showing a pronounced right-hemisphere dominance in all dyslectics, in contrast to the left-hemisphere dominance observed in 5 of 7 normal subjects. The occipital areas remained active up to 800–1000 ms. Concrete words elicited strong left frontotemporal activation after 500 ms in normals, but much weaker signals in dyslectics. Dyslexia may thus be associated with abnormal right-hemisphere dominance of early cortical analysis and reduced post-lexical activation.

695.2

HIGH-FREQUENCY OSCILLATIONS INDICATE CELL-ASSEMBLY BINDING IN HUMANS. N. Birbaumer*, W. Lutzenberger & F. Pulvermüller. Institute of Medical Psychology and Behavioral Neurobiology, Univ. Tuebingen, D-72074 Tuebingen

EEG- and MEG-synchronized responses in the range of 20 to 45 Hz indicate „binding" of cell-assemblies because they reliably appear at the human neocortex if meaningful information is processed. Three experiments with healthy subjects (N=15 in each experiment) used (a) meaningful words and meaningless pseudowords carefully matched for word length and frequency, (b) words with motor and sensory content and, (c) regular Gestalt-like and irregular visual patterns presented in different visual fields. Changes in EEG power from multiple electrode arrays were analyzed using currenct source densities based on spherical spline interpolation in order to eliminate artificial influence from reference electrodes. Meaningful words elicit 30Hz gamma responses in the left perisylvian cortex only, meaningless words showed no synchronized responding in any frequency range (3,5-100Hz). Words with motor content elicit 30Hz responses in left frontal regions, words with visual content 35Hz responses in both parietal-occipital cortices. Changes from chaotic to Gestalt-like visual patterns elicit 40Hz oscillations in the respective cortical representation of the visual field of the occipital lobe. The results cannot be explained by effects of harmonics of changes in alpha activity.

Supported by the German Research Society (DFG).

695.3

CORTICAL CONTROL OF VOCAL FUNDAMENTAL FREQUENCY DURING SINGING. D.W. Perry*, R.J. Zatorre, and A.C. Evans, Montreal Neurological Institute, McGill University, Montreal, QC, Canada H3A 2B4.

During singing, one must consciously control vocal fundamental frequency in order to produce intended musical pitches. In the present experiment, 13 subjects performed 3 experimental tasks, and changes in CBF were measured by positron emission tomography using the water bolus method. In the singing condition, they vocalized a single pitch repetitively on the syllable "ah", holding each note as long as they could comfortably on a single breath. In the playback condition, they listened to a recording of their singing. In a whisper-tracking condition, they listened to the same recording, while whispering "ah" at the same rate. In comparison to playback, significant increases in CBF were observed during singing in the supplementary motor area (SMA), the precentral gyrus, the mid-insula/precentral operculum, and the cerebellum. Increases were also observed in the right primary auditory region, and bilaterally in occipital cortex. These findings replicate those observed previously during singing in comparison to perception of complex tones (Perry *et al.*, 1993, *Soc. Neurosci. Abstr.* 19, 843). In comparison to whisper-tracking, increases during singing were observed only in the inner face of the precentral operculum, the right primary auditory region, and the cerebellum. In comparison to playback, increases during whisper-tracking were observed in SMA, the precentral gyrus, and the mid-insula/operculum. These results suggest that paramotor cortex in the inner face of the precentral operculum may be particularly crucial to the cortical control of vocal fundamental frequency, i.e. for adjustments of vocal fold length by the intrinsic laryngeal musculature. They also provide further confirmation for the hypothesis that right auditory cortex may be preferentially involved in monitoring the pitch of one's own voice during singing.

695.4

THE LEFT FRONTAL OPERCULUM IN PHONOLOGICAL PROCESSING: CONVERGING EVIDENCE FROM PET AND THE LESION METHOD. JA Fiez*, H Damasio, D Tranel, Div Cog Neurosci, Dept Neurol, U Iowa, Iowa City.

PET studies in normal subjects have provided evidence that a region at the junction of the inferior frontal gyrus and the anterior insula is important for specific types of phonological processes (1,2,3,4). Here, we report converging evidence on this finding using the lesion method. Eleven target subjects with circumscribed left frontal opercular lesions were identified in our patient registry. The target subjects and 11 normal controls matched for age, sex, and education were studied using tasks derived from the PET studies in normals. We hypothesized that the target subjects would be impaired on tasks associated with functional activation in the left frontal opercular region, but would perform normally on tasks not associated with activation of the region. Relative to the controls, the target subjects were: 1) impaired at reading nonwords (p=.004) and low-frequency words with exceptional orthographies (e.g., chassis) (p=.006), but read other words within normal limits (p>.05); 2) impaired on a phonological detection task (does a word contain a long vowel sound, e.g. the 'a' sound in LAME) with both visual (p=.002) and auditory (p=.02) presentation, but were normal (p>.05) on an orthographic detection task (does a word contain an "ascending" letter, e.g. an 'l') with both visual and auditory presentation; 3) impaired at remembering 5 words for 60 s (p=.05). The target subjects were normal on a rhyming task (p>.05). These findings provide strong additional evidence that regions within the left frontal operculum are part of a system that makes specific and critical contributions to phonological processing.

1. Fiez et al., J Cog Neur, in press; 2. Fiez et al., Soc Neurosci Abstr 19(1993):1808; 3. Petersen et al., 1(1989):153-170; 4. Raife et al., Soc Neurosci Abstr, 18(1992):932.

695.5

COMPARISON OF CORTICAL AREAS ACTIVATED BY PRIMARY AND SECOND LANGUAGES IN HUMAN BRAIN USING FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI). K. Kim*[1,3], J. Hirsch[1,3], R. DeLaPaz[2,3], N. Relkin[3], and K-M. Lee[1,3]. Depts. of Neurology[1] and Radiology[2], MSKCC; Dept. of Neurology and Neuroscience[3], Cornell University Medical College, New York, NY 10021

Fluent second languages can be acquired throughout life, providing primary language acquisition has taken place during childhood. To better understand the brain mechanisms involved in the development of multilingual capabilities after childhood, we used fMRI techniques to study the cortical regions activated during performance of identical tasks in volunteers who learned fluent English during early adulthood. Functional brain images were obtained using a GE Signa 1.5 Tesla scanner with echo-planar capability (Advanced NMR) using a gradient echo sequence with an in-plane resolution of 1.56 x 1.56 mm^2. Sixteen 4.7 mm axial slices parallel to the AC-PC line were spaced 1.6 mm apart to image virtually the entire cerebral cortex. Twice over a period of month, each subject underwent fMRI scanning as they read text, read verbally-encoded numbers and performed calculations using verbally-encoded numbers in Korean and English. A voxel-by-voxel, multistage statistical analysis with a replication requirement identified the locations of active sites associated with all tasks. Cortical regions thought to be involved in calculation showed identical activations irrespective of the language employed However, within the regions generally considered language-related (presumably Broca's and Wernicke's areas), adjacent areas of activation were reproducibly observed which appeared to be unique to Korean and English respectively. These results suggest that the mapping of primary and secondarily-acquired languages may be spatially resolvable at the level of the cerebral cortex when the second language is acquired after childhood. This could reflect recruitment of neighboring cortical zones or subdivision of language-specialized cortex in the process of acquiring a second language.

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695.6

A FUNCTIONAL MRI STUDY OF WRITING *V. Menon*, J. E. Desmond, K. O. Lim, J. B. Demb, D. Spielman and A. Pfefferbaum* Departments of Psychiatry and Behavioral Sciences, Radiology and Psychology, Stanford University School of Medicine and Palo Alto VA Medical Center, CA 94304.

Brain areas activated during writing were investigated using functional MRI. The task consisted of 6 cycles of 30s rest and 30s writing intervals. In the writing interval, subjects wrote short sentences (aphorisms) dictated binaurally; the auditory presentation typically lasted less than 5s. Subjects rewrote the sentence if they completed writing before the end of the 30s interval. Four right-handed subjects (2M, 2F) were studied. Functional images were acquired on a 1.5T scanner using a T2* weighted gradient echo spiral pulse sequence (TR=75ms, TE=40ms, flip angle=30°). Twelve sagittal slices were collected, six in each hemisphere, with slice thickness of 5mm and inter-slice separation of 2.5mm. Time-series cross-correlation methods were used to determine the loci of activation. In the left (contralateral) hemisphere, in addition to the pre-motor (BA 6), motor (BA 4) and somatosensory (BA 3, 2 and 1) cortices, activations were observed in Heschl's gyrus (BA 41 and 42), posterior superior temporal gyrus (STG, posterior BA 22) and in the frontal operculum (BA 44). Activations were also observed in the left anterior STG in 3 subjects and in the left posterior inferior temporal gyrus in 2 subjects. Strong activation was consistently observed in the left supramarginal gyrus (BA 40) of all four subjects. Little or no activation was observed in the right (ipsilateral) hemisphere, except for the cerebellum, where stronger and more widespread activation was observed in the right hemisphere. Given that the auditory stimuli typically lasted less than 5s in a cycle, the results suggest that a feedback loop between Broca's and Wernicke's areas may be involved in maintenance of and access to the phonological codes. The results are in good agreement with lesion studies implicating the left supramarginal gyrus in phonological agraphia. *Supported by The Sinclair Fund, NIH (MH30854, F32BS09628 and AG11427) and the Department of Veterans Affairs.*

695.7

ACTIVATION OF LEFT SUPERIOR TEMPORAL SULCUS WITH FUNCTIONAL MRI OF SENTENCE PROCESSING. S. L. Small[1*], D. C. Noll[2], C. A. Perfetti[3], and W. Schneider[3] Departments of [1]Neurology, [2]Radiology, and [3]Psychology; University of Pittsburgh, Pittsburgh, PA 15261.

We used functional MRI to investigate the unique neuroanatomical processing characteristics of sentences. Five right handed subjects participated. Word sequences were presented one word at a time ending with a period. Subjects pressed a button at the end of each grammatical meaningful sentence. In the control task, sequences of false font strings were presented one string at a time, with a button press at the end of each sequence. Button presses were performed with the left hand and were equalized across conditions.

Within an oscillatory stimulus presentation paradigm, active and control tasks were alternated for 30 sec each for 4 min. Six parasagittal spiral k-space images were obtained in each hemisphere with a 1.5 Tesla scanner using a pair of parallel 5-inch temporal surface coils. The head was restrained with a bite bar or a clamp. A gradient echo pulse sequence used 4 interleaved spirals to provide 2.7 x 2.7 mm resolution over an 18 cm FOV. Each slice was acquired 8 times during each 30 sec interval (total 64).

The activation waveform for each voxel was cross correlated ($r = 0.5$) with a reference (sine) waveform corresponding to the temporal pattern of stimulus presentation. The main regions of activation were the left inferior frontal gyrus and superior temporal sulcus (STS). Most subjects had a smaller degree of activation in homologous regions of the right hemisphere. Some subjects also had activation in the left angular gyrus or premotor cortex. The focus of activation in the left STS, about two thirds of the distance from the temporal tip to the temporo-occipital junction, is not identical to the region of the superior temporal gyrus implicated in most lesion studies as important for comprehension. *(Supported by NIH DC00054)*

695.8

EFFECTS OF DIFFICULTY ON FUNCTIONAL MRI ACTIVATION DURING A COGNITIVE TASK. RK Shin, M D'Esposito, JA Detre, GK Aguirre, M Grossman*, DC Alsop. Depts. of Neurology & Radiology, University of Pennsylvania Medical Center, Philadelphia, PA 19104.

The effects of increasing difficulty of a cognitive task on functional brain activation have not been well studied. Specifically, it has been argued that prefrontal activation in certain cognitive tasks (e.g. working memory tasks) may be due to mental effort alone. If this hypothesis is true, then a cognitive task that does not recruit prefrontal cortex might be expected to do so when made more difficult. We studied 10 normal subjects using echoplanar functional MRI during a spatial location/mental rotation task in which the subjects were required to match a target figure to one of two spatially rotated figures. Difficulty was varied for each subject either by increasing the number of mental rotations required to solve the task (n=6) or by increasing the presentation rate of the stimuli (n=4) until average subject accuracy decreased from approximately 90% to 70%. Scanning was performed at 1.5 T over 16 contiguous 5mm axial slices (TR=2000 msecs, TE=50 msecs). Regions of activation were identified by correlation with task above a threshold r value, and signal change was assessed within regions of interest. Task-correlated signal change was found in bilateral superior parietal lobule (Brodmann's area 7) and bilateral occipital cortex (Brodmann's area 19) in all subjects. As task difficulty increased, an increase in percentage signal change and activation volume was seen in these regions. Activation in prefrontal cortex was not noted. These findings provide insight into how the brain responds to increasing task difficulty and do not support the hypothesis that mental effort alone results in the prefrontal cortical activation seen in working memory tasks.

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695.9

AN EXPLANATION OF CONSCIOUSNESS. Eugene M. Brooks, M.D.*
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Consciousness is postulated to be a rapid sequence of multiple, discrete events which give the impression of being a single entity. It is based on "qualitative cores" which are described as the intra or intercellular aspects of nerve cells. The cores constitute the unique essences of the "five senses", the emotions, and other forms of consciousness. Nerve impulses from these cells progress "upward" and are processed. Processing includes monitoring, selecting, screening, and combining under the control of higher centers. The higher centers are driven by pleasure or displeasure in conjunction with previously formed hierarchies. Hierarchies "compete" for the attainment of consciousness and are stored in memory. Mechanisms for rapidity of action, including "synecdoche" and "abstracting" are proposed.

Nerve impulses do not create consciousness. They are consciousness experienced from the "inside." The physical or chemical elements which produce consciousness may already be known but are not recognized as such because as observers we are on the "outside." It becomes clear that theories of consciousness must be based on physical or chemical elements but until that level of explanation is reached it is fitting to rely on an anthropomorphism (a homunculus). The present paper applies not only to consciousness but also to perception, meaning, attention, curiosity, and a form of learning.

CIRCUITRY AND PATTERN GENERATION

696.1

CENTRAL GENERATION OF DIRECTED LIMB MOVEMENTS IN LOCUSTS. A. Berkowitz* and G. Laurent, Division of Biology, 139-74, Caltech, Pasadena, CA 91125.

The degree to which insect limb motor control requires movement-related sensory feedback has been debated for decades, on the basis of experiments on locomotion. We developed an insect preparation to study the neural control of scratching. We recorded fictive motor patterns for scratching by the middle leg and hindleg of locusts.

Locusts with the connectives cut between the prothoracic and mesothoracic ganglia used the middle leg or hindleg to scratch each of several sites that were stimulated mechanically. Thus, the head ganglia are not required for scratching. The hindleg could scratch 1) the ear, 2) the ventral or posterior hindleg coxa, and 3) the posterior abdomen. The middle leg could scratch the anterior hindleg coxa. The 3 types of hindleg scratching were readily distinguished in plots of tibial extension versus femoral elevation. Electromyographic recordings demonstrated that during scratching, in contrast to walking, tibial extensor muscle activity occurred partly during trochanteral levator muscle activity.

In several animals, we cut all nerve branches that provide motor innervation to the middle legs and hindlegs, while leaving intact the sensory innervation of the ear, abdomen, and most of the hindleg coxa. These animals could produce coordinated bursts of activity in tibial extensor and trochanteral levator and depressor motoneurons, in response to mechanical stimulation of the same sites that evoked scratching in animals that could move their legs. Thus, basic motor patterns for locust scratching were generated in the absence of leg movement. These central motor patterns also displayed clear intersegmental coordination between the mesothoracic and metathoracic ganglia. *Supported by an NIH NRSA to A.B. and an NSF PFF to G.L.*

696.2

MODULAR ORGANIZATION OF RHYTHM GENERATING CIRCUITRY FOR ROSTRAL SCRATCHING IN THE HEMISECTED HINDLIMB ENLARGEMENT OF THE SPINAL TURTLE. Paul S.G. Stein*, John C. Victor, and Edelle C. Field, Dept. Biology and Movement Science Program, Washington Univ., St. Louis, MO 63130.

Fictive rostral scratching is produced in a spinal, immobilized turtle by gentle mechanical stimulation of the ipsilateral (ipsi) midbody shell bridge (J. Neurophysiol. 53: 1517, 1985). Ipsi scratching motor output normally includes bursts of hip flexor motor activity that rhythmically alternate with bursts of hip extensor motor activity. Occasionally, in some cycles, "hip-extensor deletions" occur; during this spontaneously occurring variation of fictive rostral scratching, there is no quiescent period between the end of one hip flexor burst and the beginning of the next hip flexor burst. Stein et al. (J. Neurosci. 15[6]: in press, 1995) described fictive rostral scratching motor rhythms generated by a spinal, immobilized turtle with the left halves of 6 spinal segments removed. In this hemisected hindlimb enlargement preparation, the left halves of the 5 hindlimb enlargement segments (D8-S2) and the left half of the D7 segment are removed. In this preparation, ipsilateral shell bridge stimulation usually elicits ipsi rostral scratching with hip-extensor deletions, i.e., rhythmic bursts of ipsi hip flexor activity; contralateral shell bridge stimulation elicits rhythmic bursts of ipsi hip extensor activity. We add to these findings with the hemisected hindlimb enlargement preparation by using simultaneous bilateral stimulation of mirror-image locations, one in the left rostral scratch receptive field and the other in the right rostral scratch receptive field. Bilateral stimulation in this hemisected hindlimb enlargement preparation elicited rhythmic alternation between hip flexor bursts and hip extensor bursts; this is similar to the normal rostral scratching motor pattern elicited by unilateral stimulation in the preparation with an intact hindlimb enlargement. We obtained a similar result in the absence of neuromuscular blockade with kinematic analysis of hindlimb movements in a spinal hemisected hindlimb enlargement preparation. These observations support the concept of modular organization of hip flexor and hip extensor rhythm generators for rostral scratching in the turtle spinal cord. Supported by NIH Grant NS30786 to PSGS.

696.3

PURINERGIC MODULATION OF SWIMMING MOTOR PATTERN IN *XENOPUS* EMBRYOS. N. Dale* and D. Gilday. School of Biological & Medical Sciences, University of St. Andrews, KY16 9TS, U.K.

Although sensory inputs evoke and modify motor activity in the *Xenopus* embryo, the motor pattern for swimming follows an inherent dynamic progression in their absence. The frequency of swimming gradually falls throughout an episode until activity spontaneously ceases. The mechanisms underlying these time-dependent changes remain unknown.

Applications of the P2 receptor antagonist, pyridoxal-phosphate-6-azophenyl-2',4'-disulphonate (10μM), shortened swimming episodes by 84±7% (±sem, n=4), suggesting that ATP may be released during fictive swimming. Adenosine applied at 10-100μM diminished the length of swimming episodes by 83±3% (n=3), while the A receptor antagonist, 8-phenyltheophylline (2.5μM), enhanced their length by 106± 21% (n=6). Use of α,β-methylene-ADP, to block conversion of AMP to adenosine by ectonucleotidases, also lengthened swimming episodes (by 262±150%, n=5). Naturally released ATP may thus be hydrolysed to adenosine which could then oppose the actions of ATP during fictive locomotion.

The mechanisms underlying these effects of ATP and adenosine have been investigated both in the intact spinal cord and in neurons acutely isolated from the spinal locomotor circuitry. Neither ATP nor adenosine appeared to: 1) gate an ion channel directly; or 2) cause presynaptic inhibition. However, whole cell patch clamp recordings showed that ATP reduced voltage-gated K^+ currents by 16±1.6% (n=11), while adenosine reduced voltage-gated Ca^{2+} currents by 14±3.2% (n=6).

Within the spinal motor circuitry, release of ATP may enhance excitability during fictive swimming by reducing K^+ currents whereas delayed formation of adenosine, by ectonucleotidases, may do the opposite by gradually reducing Ca^{2+} currents. Time-dependent modulation of voltage-gated currents by ATP and adenosine may thus underlie the dynamic regulation and self-termination of the swimming motor pattern.

696.4

GENES THAT CONTROL A MOTOR PROGRAM PERIOD IN *C. ELEGANS* K. Iwasaki*, D. WC Liu, and J. H. Thomas, Dept. of Genetics, Univ. of Washington, Seattle, WA 98195

Periodic activation of a motor program is controlled by a neuronal pattern generator. Electrophysiology of some neuronal pattern generators, such as in *Tritonia* and *Panulirus*, has been extensively studied. However, the genetic basis of such pattern generators is not understood. Recently, we have found that *C. elegans* defecation is controlled by a temperature-compensated clock, possibly a neuronal pattern generator. With the exception of its periodicity (45 seconds), various properties of this clock are similar to those of a circadian clock. The defecation motor program consists of a stereotyped series of three muscle contractions, called pBoc, aBoc, and Exp. To investigate how the defecation cycle period is genetically controlled, we screened approximately 3,000 mutagenized haploid genomes and reexamined existing mutants. We identified mutations in 12 genes can cause abnormal defecation cycle periods. These defecation cycle periodicity (Dec) mutations fall into two major groups, short cycle (Dec-s) and long cycle (Dec-*ℓ*). Detailed characterization of these mutants revealed the following points. 1) Most Dec mutations affected the cycle period quite differently at 20° and 25°. Because of their high frequency of isolation, we think most of the mutations affect temperature compensation rather than causing thermolabile gene products. 2) Mutations in the *flr-1*, *flr-3*, and *flr-4* genes (Katsura, *et. al.* Genetics **136** 145) showed a very short mean cycle period. The short cycle period correlated strongly with weakened pBoc and Exp motor steps, suggesting depletion of a factor by a high frequency of motor program activation. 3) Dec-s mutations in *dec-9* and *dec-10* were dominant. These mutations were gain-of-function based on heterozygous deficiency phenotypes. 4) Most of the *dec* mutations did not affect internal timing of the motor program. However. mutations in two Dec-*ℓ* genes, *dec-2* and *dec-4*, lengthened the interval between pBoc and Exp steps.

We are currently building double mutants among the Dec mutations in order to construct a genetic regulatory pathway for the clock.

696.5

THE CIRCUIT LOCUS OF THE BEHAVIORAL CHOICE BETWEEN SWIMMING AND SHORTENING IN THE LEECH. B. K. Shaw* and W. B. Kristan, Jr. Dept. of Biology, Univ. of California, San Diego, La Jolla, CA 92093-0357.

We are studying the neuronal basis of the behavioral choice between two incompatible behaviors in the leech, swimming and the whole-body shortening reflex. An advantage of studying these behaviors is that the neural circuit for swimming is particularly well understood. It is made up of a hierarchy of identified neurons: head brain interneurons influence segmental swim-gating interneurons, which activate the oscillator interneurons that make up the CPG for the rhythym, which in turn drive the motor neurons.

At a behavioral level, the shortening reflex dominates swimming: swimming is prevented during shortening and shortening can interrupt swimming. In correspondence with this at a neuronal level, the cells 204— the most powerful of the swim-gating interneurons—are strongly inhibited during shortening. The cells SE1, 'swim excitor' interneurons in the brain which strongly excite the cells 204, show an opposite effect: they are excited during shortening. This means that during shortening, the cells SE1 can have an inappropriate excitatory effect on the cells 204 that must be overridden by other, inhibitory, inputs to 204. This suggests that the cells 204 act, at least in part, as the 'decision-site' where the signals that promote and suppress swimming are integrated. It also suggests that the cells 204 are examples of interneurons that are dedicated to a single behavior—swimming—while the cells SE1 may be multifunctional interneurons that participate in more than one behavior. To further test these hypotheses, we will record from other swim circuit interneurons, like the oscillator cells, to determine their role in shortening. Supported by NIGMS training grant GM08107 (BKS) and NIMH research grant MH44396 (WBK).

696.6

NEURONAL MECHANISMS MEDIATING CONTEXT-SPECIFIC COMMANDS IN THE FEEDING SYSTEM OF *APLYSIA*. S. C. Rosen*, E. C. Cropper[1], F. S. Vilim[1], K. R. Weiss[1] and I. Kupfermann. Cntr. Neurobiol. & Behav., NYS Psychiat. Inst., New York, NY 10032; [1]Dept. Physiol. & Biophys., Mount Sinai Sch. of Med., New York, NY 10027.

Appropriate behavior depends in part on an animal's ability to initiate the behavior within a complex context. Neuronal mechanisms mediating context-specific initiation of behavior were investigated in the *Aplysia* feeding system where command-like interneurons have been identified and where initiation of ingestion is dependent upon: (1) the generation of a postural state associated with food-induced arousal; (2) a combined chemical and tactile stimulus applied to the lips; and (3) a reinforcement state produced by the success of previous responses. Three types of excitatory input to biting command-like interneuron CBI-2 may carry state-dependent information: (1) input from neuron CPR that controls the postural arousal system; (2) combined chemosensory and mechanosensory input produced by food contacting the peri-oral zone; and (3) direct input from a CPG interganglionic projection interneuron (B19) that is excited by mechanoafferent neurons innervating the grasping surface of the radula. B19 was found to produce a conductance decrease slow EPSP in CBI-2 that can amplify other inputs to the cell. Since CBI-2's resting potential is 15-25 mV below threshold and because CBI-2 must fire above 10 Hz to effectively drive a rhythmic motor program, it is likely that two or more inputs must act in concert to produce robust behavior. The results indicate how crucial logical operations may be executed by command-like neurons.

696.7

A MOTOR PROGRAM ASSOCIATED WITH FEEDING IN ISOLATED CEREBRAL GANGLIA OF *APLYSIA*. Ray Perrins* and K. R. Weiss Dept. Physiology and Biophysics, Mount Sinai School of Medicine, New York, NY 10029.

The coordination of mouth and buccal mass movements varies in different forms of feeding-related behavior in *Aplysia* and indeed can occur independently. Buccal motor programs (BMPs) controlling buccal mass movements can be obtained in isolated buccal ganglia so the central pattern generator (CPG) controlling this aspect appears to reside there. Mouth movements are controlled largely by motoneurons in the cerebral ganglion and we have now discovered a neuron, the cerebral motor program initiator (C-MPI), which can induce a cerebral motor program (CMP) in the isolated ganglion when fired at rates of 12-26 Hz. The cycle period of the program is between 9 and 50 seconds, similar to those observed for BMPs. This program incorporates alternate firing of lip motoneurons C11 and C12 as well as e-cluster neurons including C4 and C-MPI itself. The cerebral to buccal interneurons CBI-2 and CBI-4, which can induce BMPs fire rhythmically during the CMP. When the buccal ganglion is attached the CMP can occasionally evoke single cycles of a BMP but more commonly has little effect on buccal output. During BMPs evoked by firing in CBI-2 and CBI-4, C-MPI receives strong rhythmic excitatory input. Thus there are CPGs in both cerebral and buccal ganglia which can control individual motor programs without the need for input from the other. However, the CPGs are connected at several sites. Altering the strengths of these connections, or decoupling the CPGs altogether may provide a mechanism for varying the coordination of mouth and buccal mass movements in different behaviors.

696.8

USING RNA-PCR TO DETERMINE LEVELS OF *SHAKER* FAMILY GENE EXPRESSION IN NEURONS AND GLIAL CELLS IN THE STOMATOGASTRIC GANGLION OF THE SPINY LOBSTER. D.J Baro*, C.L. Cole, H.E. Rodriguez, and R.M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14850.

The unique electrophysiological phenotypes of the different identified neurons in the stomatogastric ganglion (STG) are essential for the generation of the pyloric motor pattern. To better understand the roles of identified currents in motor pattern generation, we are trying to correlate specific cellular currents with specific genes from the *Shaker* family of potassium channels. As a first step toward this end, we have used RNA-PCR to survey unidentified neurons in the STG to determine what fraction of the neurons detectably express a given *shaker* family gene. In these experiments individual neurons serve as templates in RNA-PCRs. Our primers are specific for the four members of the lobster *Shaker* family genes and an alpha-tubulin gene, and always flank an intron to ensure that we are detecting RNA and not chromosomal DNA. Each gene appears to be expressed in at least 63% of the neurons surveyed. We have also used this method to determine which genes are detectably expressed in glial cells. Thus far, we have only been able to detect *shal* gene expression in glial cells. As the next step toward our goal, we have developed and are currently using a quantitative RNA-PCR method to measure differences in the level of *Shaker* family gene expression in identified neurons of the pyloric network. Supported by NIH NS25915.

696.9

DOPAMINE (DA) AND SEROTONIN (5HT) COORDINATE DIFFERENT ASPECTS OF CONSUMMATORY FEEDING BEHAVIOR IN *APLYSIA*. E.A.Kabotyanski*, D.A.Baxter and J.H.Byrne. Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77030.

One of the salient features of biological pattern generators is their ability to be modulated by extrinsic and intrinsic factors. The mechanisms by which this modulation occurs are poorly understood however. We have begun to examine this issue using feeding behavior in *Aplysia*. This behavior is controlled partially by the buccal ganglia. Bath application of DA induces patterned neural activity in isolated buccal ganglia and biases this activity toward ingestion-like motor programs (Baxter *et al.*, this volume). Although 5HT has been implicated in the enhancement of consummatory responses (Rosen *et al.*, 1989), it has also been shown to inhibit aspects of motor programs in isolated buccal ganglia (Baxter *et al.*, this volume). We have developed a semi-intact preparation to provide direct behavioral evidence correlating feeding behaviors with the actions of DA, its precursor DOPA, and 5HT.

The preparation consisted of the isolated anterior portion of the animal with attached CNS. The canulated buccal artery was perfused with saline to apply drugs and appropriate hydrostatic pressure. The force produced by foregut movements was monitored isotonically with a transducer attached to a thread that was drawn through the mouth and esophagus. In control saline, low frequency (~0.006 Hz) feeding-like movements occurred with no net displacement of the thread. DA (50 μM) evoked frequent (~0.04 Hz) cyclical opening-closing of the jaws and protraction-retraction of the radula, which appeared similar to bites in intact *Aplysia*. These movements led to inward displacement of the thread. Effects of DOPA (250 μM) were similar. 5HT (5 μM) did not significantly change the frequency of foregut movements, but each cycle resulted in a large inward displacement of the thread. 5HT-induced movements, most of which occurred without jaw openings, were distinct from DA-induced movements and appeared similar to swallows (Morton & Chiel, 1993; Kupferman, 1974).

Our results indicate that DA and 5HT may be involved in the control of ingestive consummatory feeding. In particular, DA appears to coordinate the generation of biting, whereas 5HT appears to coordinate the generation of swallowing.

696.10

CHARACTERIZATION OF MOTOR PROGRAMS GENERATED IN ISOLATED BUCCAL GANGLIA OF *APLYSIA* AND THEIR MODULATION BY BIOGENIC AMINES. D.A. Baxter*, S.J. Cushman, E.A. Kabotyanski and J.H. Byrne. Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77030

To provide further insights into the coordination of motor patterns, we monitored patterned neural activity in isolated buccal ganglia with extracellular and intracellular recordings. Based on similarities to previous recordings from freely behaving animals and on the phase relationship between activity in identified motor neurons (e.g., Morton and Chiel 1993; Church and Lloyd 1994), three patterns were identified: a rejection-like pattern, an ingestion-like pattern, and an intermediate pattern. In control saline, all three occurred spontaneously. However, the rejection-like pattern was expressed most often and the ingestion-like pattern was the least common. In addition, stimulation of the radula nerve usually elicited the rejection-like pattern.

Bath application of dopamine (DA; 50 μM) had three effects. First, DA increased the occurrence of spontaneous patterned neural activity. Second, DA increased the number of ingestion-like patterns and decreased the number of rejection-like patterns. Third, in the presence of DA, stimulation of the radula nerve elicited an ingestion-like pattern rather than a rejection-like pattern. The effects of serotonin (5HT) were complex. First, 5 μM 5HT decreased the occurrence of spontaneous patterned neural activity. In contrast, 0.5 μM 5HT increased spontaneous patterned activity. Neither concentration of 5HT significantly altered the relative distribution of the types of motor patterns, however. Second, in the presence of 5 μM 5HT, stimulation of the radula nerve failed to elicit patterned neural activity. Finally, 5 μM 5HT attenuated the DA-induced patterned neural activity.

These results, and those of others, indicate that the buccal ganglia contain a central pattern generator (CPG), which in isolation can produce patterns of neural activity similar to those observed in intact and semi-intact preparations, and that biogenic amines play a role in determining the functional configuration of the CPG. For example, DA induced ingestion-like activity, which is a likely neural correlate of biting (Kabotyanski et al. this volume). 5HT also has been shown to enhance aspects of ingestive behaviors. For example, 5 μM 5HT induced swallowing-like movements in our semi-intact preparation. Neural correlates of such 5HT-mediated enhancement were not apparent in isolated buccal ganglia, however. One possibility is that these correlates of 5HT may arise from more complex integration by additional central and/or peripheral neural elements.

696.11

AFFERENT CONTROL OF BUCCAL MOTOR PROGRAMS IN *APLYSIA*. R. Nargeot*, D.A. Baxter and J.H. Byrne. Department of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77030.

Behavioral studies have shown that the central pattern generator (CPG) underlying consumatory feeding behavior in *Aplysia* can mediate, in response to different types of sensory input, either ingestion or rejection. We have begun to examine how afferent inputs to this CPG influence its activity in a reduced preparation.

The reduced preparation consisted of isolated buccal ganglia with two of its peripheral nerves (see below) still attached to small patches of the inner surface of the buccal mass. Motor nerve activity was monitored with extracellular recordings. Activation of afferents could elicit feeding motor programs in previously quiescent preparations. A motor program, which has previously been demonstrated to mediate ingestion in intact and semi-intact preparations (Morton & Chiel, 1993a,b) was induced by applying seaweed to the inner surface of an anterior portion of the buccal mass. In contrast, no motor activity was induced by a similar stimulation of a posterior portion of the inner surface of the buccal mass. One or two cycles of a rejection-like pattern could be elicited by stretching either portion of the buccal mass.

The axons of the afferents implicated in these responses were located in the most anterior branch of the buccal nerve 2 (which we refer to as BN2,3) and the anterior branch of the esophageal nerve (which we refer to as EN2). Rhythmic motor activity composed of both ingestion- and rejection-like patterns was induced by tonic electrical (2Hz) stimulation of BN2,3, whereas tonic electrical stimulation of EN2 only induced a rejection-like pattern. In all cases, the induced rhythmic activity lasted as long as the tonic stimulation was applied, whereas phasic electrical stimulation (10Hz, 6s) of either nerve evoked only one or two cycles of a patterned motor activity.

These results indicate that in isolated buccal ganglia, afferent inputs can induce patterned motor activity similar to that observed in freely behaving animal (e.g., ingestion and rejection). Moreover, the finding that a single afferent pathway (e.g. BN2,3) could elicit two distinct motor programs during tonic activation, suggests that the CPG can undergo rapid reconfiguration, which may underlie behavioral switching. Such a reduced preparation will be suitable for investigating the cellular mechanisms contributing to such reconfiguration.

696.12

INTERPLAY OF EXCITATORY AND INHIBITORY PATHWAYS DURING NEUROCHEMICALLY INDUCED LOCOMOTOR ACTIVITY IN THE NEONATAL RAT SPINAL CORD. E. Kremer and A. Lev-Tov*, Dept. of Anatomy, The Hebrew University Medical School, Jerusalem, Israel.

The left-right alternation of neurochemically induced locomotion was studied in the isolated spinal cord preparation of the neonatal rat using wide-band AC extracellular recordings from lumbar ventral roots and intracellular recordings from segmental motoneurons. The left-right alternation of the locomotor rhythm could be converted into bilaterally synchronous bursts by bath application of the glycine receptor blocker strychnine, the $GABA_A$ receptor antagonists bicuculline and picrotoxin and by the nicotinic acetylcholine receptor blockers hexamethonium and mecamylamine. The drug induced bilateral synchronicity was not unique to the lumbar cord and occurred in most segments of the spinal cord with intact or detached lumbar segments. When occurred, the synchronous bursts could be transmitted rostrally and caudally by ascending and descending pathways traveling in the ventral, the ventrolateral the lateral and dorsolateral funiculi. Reduction of the enhanced excitatory drive of the cord by pre-treatment with the excitatory amino acid receptor blockers CNQX or AP5, by bath application of the GABA uptake inhibitor nipecotic acid, and in some cases by the use of mid-saggitally split spinal cord preparations with a single bilaterally-intact upper lumbar segment, unmasked left-right alternating rhythm in the presence of either bicuculline, strychnine or the nicotinic cholinergic antagonists. It seems that the idea of relating the drug induced "conversion" of the rhythm to a blockade of reciprocal inhibition is oversimplified. We suggest that the cross excitatory pathways between the hemicords are strongly modulated by various inhibitory projections and that the efficacy of these projections may play a significant role in determination of the phase relation between the left and right hemicords during neurochemically induced locomotion.

CELL LINEAGE AND DETERMINATION IV

697.1

PURIFICATION AND CHARACTERIZATION OF ADULT OLIGODENDROCYTE PRECURSOR CELLS
J. Shi, A. M. Marinovich and B. A. Barres*. Dept. of Neurobiology, Stanford University, School of Medicine, Stanford, CA 94305-5401, USA.

Although the extent to which demyelinated white matter can repair itself is not known, the injured brain appears to have at least some ability to generate new oligodendrocytes. It has been suggested that new oligodendrocytes may arise, at least in part, from oligodendrocyte precursor cells that persist in the adult central nervous system. Oligodendrocyte precursor cells in adult brain divide little, if at all, whereas they divide rapidly during development. Similarly, it has been reported that adult precursor cells in culture divide more slowly than perinatal precursor cells. Fundamentally, such differences between the behavior of adult and perinatal oligodendrocyte precursor cells could be the result of either intrinsic or extrinsic differences (or both). In order to answer this question and to further characterize the properties of the adult precursor cells, we have directly compared the properties of highly purified perinatal and adult precursor cells. 2,000 adult oligodendrocyte precursor cells per P60 rat optic nerve were isolated by immunopanning to greater than 99% purity. The purified cells share several properties with their perinatal counterparts: nearly all differentiated into GC^+ oligodendrocytes in serum-free medium or into $GFAP^+$ type-2 astrocytes in serum-containing medium. Clonal studies showed that nearly all adult oligodendrocyte precursor cells divided symmetrically in serum-free medium containing PDGF, NT-3, IGF-1 and thyroid hormone. They divided, however, at least five to ten times more slowly than the perinatal cells and generated few oligodendrocytes. The purified adult precursor cells did not revert to rapidly dividing cells in PDGF and FGF. These studies demonstrate that perinatal and adult oligodendrocyte precursor cells are intrinsically different and raise the question of whether adult oligodendrocyte precursor cells have the ability to revert into rapidly dividing precursor cells.

697.2

THE PRESENCE OF MICROGLIA IN MURINE EMBRYONIC CULTURES OF EGF-GENERATED NEUROSPHERES. A.K. Papavasiliou, M.F. Mehler, K. Dobrenis, R. Marmur, P. Mabie, C.S. Raine*, J.A. Kessler. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Although microglia play a prominent role in central nervous system (CNS) pathophysiology, their relationship to other major cellular lineages during CNS development is not well characterized. The predominant view is that all microglia are of monocytic lineage. Alternatively, it is possible that during fetal development a subpopulation of microglia also arise from endogenous progenitor cells. To address this issue, we have studied the elaboration of microglia in epidermal growth factor (EGF)-generated multipotent progenitor cells from the E17 mouse subventricular zone (SVZ), known to give rise to the three major CNS lineages. Utilizing the following microglial markers, Griffonia Simplicifolia I-B4 Isolectin, dil-acetylated LDL, Mac-1 and F4/80, we now report the presence of microglia within these cultures. Treatment of serially passaged EGF-generated neurospheres with either CSF-1 or GM-CSF altered microglial morphology leading to ramified (CSF-1) or amoeboid (GM-CSF) microglia with a small increase in cell number. By contrast, treatment with microglial conditioned media greatly increased the number with both morphologies. Application of cytokines (bFGF,CSF-1, IL-6) allowed the generation of microglia from single isolated neurospheres. Current studies are underway to examine whether these microglia are derived from neural progenitor cells.

697.3

TRANSFECTION OF DISSOCIATED EMBRYONIC AND POSTNATAL RAT RETINAL CELLS IN CULTURE BY PARTICLE-MEDIATED GENE TRANSFER. E. M. Levine, L.C. Streichert*, and T. A. Reh, Department of Biological Structure, University of Washington, Seattle, WA 98195.

The difficulty in transfection of progenitor cells and neurons is a major obstacle of gene expression studies in the mammalian central nervous system. Using particle-mediated gene transfer, or biolistics, we have successfully transfected coverslip cultures of dissociated E18 - P2 rat retinal cells. In biolistics, DNA is precipitated onto micron sized gold particles that are delivered to the cells by a high-pressure burst of helium in an evacuated chamber. To determine the effectiveness of this approach, we transfected the pCS2 expression vector containing either the E.coli ß-galactosidase (ß-gal) gene or a 6-repeat myc epitope tag (MT) under the control of the immediate early CMV promoter. Cytochemical detection of ß-gal was done with the substrate X-gal or with anti-ß-gal antibodies. The MT was detected with the 9EC10 anti-MT monoclonal antibody. Transfection of high-density coverslip cultures (*initial plating density* 2.5 x 10^5 cells) ranged from a few hundred to over 1000 cells. Expression of the transfected plasmids was observed 8 hours to 9 days post-transfection. Using cell-type specific antibodies to identify the retinal cell classes, transfection was observed in most retinal neurons as well as progenitor cells. In co-transfections using the pCS2-ß-gal and pCS2-MT, most cells that were successfully transfected expressed both ß-gal and the MT. Therefore, this technique is amenable for gene expression studies in embryonic and postnatal rat retinal cultures.

This work was supported by funds from NINDS.

697.4

DETECTION OF GENOMIC SEQUENCE VARIATION BETWEEN NEOCORTICAL CELL LINES AND GERM LINE DNA. J.J.A. Contos and J.J.M. Chun*. Dept. of Pharmacology, School of Medicine, University of California at San Diego, La Jolla, CA 92093-0636

We are investigating the hypothesis that genomic DNA rearrangements occur during the normal development of mammalian CNS neurons. The technique we have utilized is representational difference analysis (RDA), a PCR-based method that allows selective amplification of low molecular weight restriction fragments unique to a given DNA sample (Lisitsyn et. al., Science 259: 946, 1993). With the assumption that the cells of the CNS are not homogeneous with respect to genomic DNA variability, a clonal cell line derived from the mouse embryonic telencephalon was used to derive DNA representing potential rearranged loci in the CNS. RDA was used to amplify differences between this genomic DNA and putative germ-line genomic DNA (derived from adult liver). The cell line contains one copy of a retroviral provirus with the SV40 Large-T oncogene (LgT). A *HindIII* fragment of 527 bp in the LgT gene, not present in the liver genomic DNA, serves as a positive control for the technique. Southern blot analysis of RDA products has shown this fragment to be selectively amplified in our experiments. Several other restriction fragments of various sizes were also selectively amplified. These RDA products may result from altered restriction fragment length created by proviral integration, spontaneous *in vitro* mutations in the cell line during culture, or normal *in vivo* rearrangements occurring before the cell line was established. Three approaches are being used to analyze these fragments: 1) identification of proviral-containing products by Southern-blot analysis, 2) determination of sequence homologies within/between various products and to known genes, and 3) generation of RDA products from independently derived cell lines to determine which products among various cell lines have common genomic loci. [supported by the March of Dimes, the California Tobacco Disease Related Program, and the Lucille P. Markey charitable trust]

697.5

A PRODUCT(S) OF ACTIVATED MICROGLIA PROMOTES CHOLINERGIC DIFFERENTIATION OF CULTURED BASAL FOREBRAIN NEURONS. G. M. Jonakait*, M. B. Luskin, R. Wei, and L. Ni. Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102 and Dept. Anatomy & Cell Biol., Emory University School of Medicine, Atlanta, GA 30322

In cultures of embryonic septal nuclei with adjacent basal forebrain (SN/BF), interferon-γ (IFNγ) dramatically increases the activity of choline acetyltransferase (ChAT) by increasing the number of cholinergic neurons (Jonakait et al., *Neuron* 12:1149). Since the target of IFNγ is ameboid microglia, we have assayed the effects of conditioned media (CM) derived from enriched cultures of activated (i.e., IFN-treated) primary microglia and from an unstimulated monocyte/macrophage cell line (RAW 264.7) on ChAT activity in septal cultures, and have found that these CM raise ChAT activity in SN/BF cultures 15-16 fold. Efficacy of CM is dependent on the density of cultured cells, reaching maximal effects at approx. 10^6 cells/ml. IFN treatment of RAW cells is not required to obtain activity.

Cultures labeled with retroviral markers (BAG) and treated with either IFN or CM from activated microglia or RAW cells show a 3-9-fold increase in the percentage of cholinergic cells detected in retrovirally labeled clones. Neither NGF nor bFGF mimics this action. These data suggest that all three treatments promote cholinergic differentiation of undifferentiated precursors present in the cultures.

697.6

PURKINJE CELL LINEAGE AND THE TOPOGRAPHIC ORGANIZATION OF THE CEREBELLAR CORTEX: A VIEW FROM X INACTIVATION MOSAICS K. Schilling*[1], B. Rosengarten[1], M.L. Schilling[1], W. Pretsch[3], S.L. Baader[1], and H.F. Teutsch[2]. 1) Abt. Anatomie & Zellbiologie, 2) Abt. Anatomie, Universität Ulm, D-89069 Ulm, Germany, and 3) Institut für Säugetiergenetik, GSF Neuherberg, D-85758 Oberschleißheim, Germany

We utilized a strain of mice, derived from a radiation mutagenesis experiment and carrying an activity-attenuated allele of the X linked enzyme, G6PD, to analyze the development of the cell lineage leading to cerebellar Purkinje neurons. Due to random X inactivation during early embryonic development, X linked genes can be used to distinguish between clonally related populations of cells in X inactivation mosaics. Following histochemical staining for G6PD activity, the numeric proportions of Purkinje cells expressing either the wild type or the mutant enzyme and the spatial distribution of these cellular phenotypes and their relation to anatomically and genetically defined cerebellar compartments was analyzed. Our data suggest that cerebellar Purkinje neurons originate from a limited pool of precursors. The size of this pool is different from the one derived from chimeric mice (e.g. Vogel & Herrup, Dev.Biol. 156, 49, '93), allowing us to deduce the relative timing of Purkinje cell lineage restriction. Our data also show that Purkinje neurons of distinct lineage are extensively intermingled within the cerebellar cortex. Together, these findings imply that genetically defined cerebellar compartments (e.g. Oberdick et al, Neuron 10, 1007, '93) are not a simple consequence of the spatial arrangement of clonally related Purkinje cells. Rather, they suggest a role for cell/cell communication in the development of these compartments and hint to a temporal window during which such cellular interactions may take place. Supported by the BMBF, the DFG, and NATO.

697.7

CELL LINEAGE AND EMBRYONIC ORIGINS OF TASTE RECEPTOR CELLS. L.M. Stone*, T.E. Finger, U. Colo. Hlth. Sci. Ctr., Denver, CO 80262 P.P.L. Tam, U. Sydney, Sydney, Australia, and S.-S. Tan, U. Melb., Melbourne, Australia.

Taste buds consist of several cell types, based on morphological and immunocytochemical characteristics. For example, basal, dark, intermediate and light cells can be distinguished using electron microscopy (e.g. Delay et al., J. Comp. Neurol., 1986); subsets of cells that are serotonin immunoreactive or gustducin immunoreactive can be distinguished at the light microscopic level (Böttger et al., Chem. Senses, in press). Using mosaic analysis, we previously demonstrated that multiple progenitors give rise to this complex population of neuroepithelial cells and that these progenitors derive from local epithelium (Stone et al., PNAS, 1995). Further investigation now has revealed that this local epithelium giving rise to taste buds may be of either ectodermal or endodermal origin. Currently, we are addressing the question of cell lineage relationships within the taste bud. As in previous studies, we are using X inactivation mosaic mice to examine clonal relationships. These mice are transgenic, female mice that contain multiple copies of a *lacZ* marker on one of their two X chromosomes. Early in development, this results in the ubiquitous production of ß-galactosidase (ß-gal) in embryonic cells. However, during development, as occurs in all mammalian females, one of the two X chromosomes is randomly inactivated in each cell. This process is stable and clonally heritable, and results in two cell populations in the mouse: one that expresses ß-gal, and one that does not. Thus the mouse is mosaic for ß-gal expression, and this mosaicism can be used for cell lineage analysis. In the taste bud, this is accomplished by analyzing taste buds that contain both ß-gal+ and ß-gal- cells. Statistical analysis of the relationships between ß-gal expression and morphological or immunocytochemical cell types indicates which of these cell types are clonally related.

697.8

HUMAN LEPTOMENINGEAL CELLS DIFFERENTIATE INTO ASTROCYTE-LIKE CELLS IN INJURED RAT SPINAL CORD. J.J. Bernstein[1]*, S.A. Karp[1], L.A DiGorgio[2] and J.P. Blass[2]. Lab CNS Regen & Neuro-Oncol, DVA Med Ctr, Wash DC, 20422[1] & Burke Med Res Inst, Cornell Univ Med Coll, White Plains, NY[2]

Human leptomeningeal cells (HLC) were cultured at autopsy from a 84 year old woman (6 hours after death). 10^6 HLC (in 4 µl), incubated in fast blue as a premarker, were placed in an aspiration pocket in the C3 dorsal columns of 40, adult, athymic male rats. Rats were perfused with 4% paraformaldehyde after 3 (day),1,2,3,4, and 8 weeks. Using confocal microscopy and immunofluorescence, studies were performed using antibodies to GFAP and neurofilament protein. Cells in the spinal cord that contained fast blue, were positive for GFAP and negative for neurofilament protein were considered to be grafted HLC that differentiated with astrocyte-like characteristics. Cells with these characteristics were found in the adjacent dorsal and ventral horns of the spinal cord in the area of the implantation pocket. These cells were observed for the 8 weeks of the experiment. These data indicate that there are pluripotential cells in aged humans.

697.9

Embryonic rat hindlimb cells injected into the caudate-putanum mature into fast and slow muscle fibres arranged as fascicles around chondrogenic cores.
C.L. Pin, W.J. Rushlow, K.A. Rogers, A.W. Hrycyshyn* and P.A. Merrifield. Dept. of Anatomy, Univ. Western Ontario, London, Ontario, Canada N6A 5C1.

Myoblasts obtained from embryonic day 14 (ED 14) rat hindlimbs have been shown in culture to differentiate into small myotubes which exclusively express embryonic and slow myosin heavy chains (MyHC). Unlike myoblasts obtained from later time points in development, these cells undergo limited fusion and do no not express fast MyHCs. (Dev. Genet. 14, 356. 1993). Due to these differences, we have postulated that "embryonic myoblasts" represent a myoblast lineage responsible for the formation of slow muscle fibres *in vivo*. To test this hypothesis, cell suspensions from ED14 rat hindlimbs were stereotaxically injected into the caudate-putanum of adult rats to determine their developmental potential in an ectopic site. At two weeks post-injection, it could be seen that extensive proliferation and fusion of the myoblasts had occurred. By four weeks, the grafts were histologically similar to fetal hindlimbs. In every graft, large muscle fascicles could be seen encircling a cartilaginous core. Prior to innervation, muscle fibres typically contained peripherally-located nuclei and expressed slow, fast or both slow and fast adult MyHC isoforms. Staining with alcian blue and silver nitrate revealed that the cartilaginous cores are undergoing osteogenesis, exhibiting all of the typical stages of endochondral ossification, and are enclosed by a well developed periosteum. These results suggest that (a) ED14 hindlimb myoblasts are capable of forming both slow and fast muscle fibres when maintained under permissive conditions *in vivo*, and that (b) innervation is not required for the expression of adult fast and slow MyHCs. Furthermore, the organization of cells within the grafts to form osteogenic cores and muscle fascicles suggests that embryonic cells sort themselves within the grafts to generate structures similar to those seen in fetal limbs. (Supported by MDAC).

697.10

EXPRESSION OF MEF2C IN FETAL MOUSE BRAIN.
D. Leifer,* Y. L. Li, and K. Wehr. Dept. of Neurology, Yale University School of Medicine, New Haven, CT 06510.

MEF2C (myocyte-specific enhancer binding factor 2C) is a member of the MADS family of transcription factors and is expressed preferentially at high levels in human and rodent cerebral cortex. We have previously studied the distribution of MEF2C immunoreactivity in human cerebral cortex from 14 weeks of gestation to adulthood. In adult human cortex, double-labeling experiments indicate that it is expressed preferentially in inhibitory interneurons. In human fetal cortex, MEF2C immunoreactivity is localized to the cortical plate. It is not found in the ventricular or intermediate zones during the stages of fetal development that we examined, but cortical neurogenesis is nearly complete by the earliest ages that we were able to examine. We have now used *in situ* hybridization and immunohistochemistry to study MEF2C expression in fetal mouse brain during the period from E11 to E17 when most cortical neurons are generated. Both methods indicate that MEF2C is expressed in the cortical plate and that little, if any, is present in the ventricular or intermediate zones. In addition, MEF2C is detectable in other areas of the brain including the olfactory bulb, the basal forebrain, the inferior colliculus, and the cerebellum, and in some cells within the thalamus and amygdala and in scattered cells elsewhere in the brain. These results indicate that MEF2C expression is tightly controlled both spatially and temporally. In addition, the results indicate that it appears to be turned on in cortical neurons after they become post-mitotic and migrate to the cortical plate. This pattern of expression suggests that MEF2C may have a role in the post-mitotic differentiation of specific subsets of neurons.

697.11

CLONING & EXPRESSION OF ZEBRAFISH LH-2/apterous HOMOLOGS.
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LIM homeo domain transcription factors play important roles in cell fate determination during development in vertebrates and invertebrates. Drosophila ***apterous(ap)***, a member of this family, is involved in the establishment of dorso-ventral compartment in wing imaginal disc and in identity determination of specific sets of muscles and neurons. Recently, ***LH-2***, a vertebrate counterpart of ***ap***, was cloned in rat, and shown to be expressed in the nervous system. We identified ***LH-2/ap*** homologs in zebrafish and examined its expression pattern using in situ hybridization. Two of them were expressed in the developing eyes and diencephalon at 10hr after fertilization, but the expression in the eyes faded by 16hr. By 28hr, their expression was restricted to many neuromere boundaries (e.g. between thalamus and pretectum, pretectum and tectum, midbrain and hindbrain) and also to every rhombomeric border in the hindbrain. Double staining with in situ hybridization and an antibody which stains most axons revealed that many early tracts of developing zebrafish brain (POC, DVDT and commissural tracts in rhombomere) were formed along the regions where ***LH-2*** mRNA was expressed. Accumulating evidences have suggested that early neurons are born along the neuromere boundaries and neuromere boundaries may express local cues for axonal guidance. The expression pattern of zebrafish ***LH-2*** suggests that LH-2 may control either the expression of such cues or the differentiation of neurons on the borders.

697.12

DEVELOPMENTAL REGULATION OF THE PDGF α-RECEPTOR EXPRESSION IN GLIAL CELLS AND NEURONES OF THE MOUSE CENTRAL NERVOUS SYSTEM.
B. Nait Oumesmar, L. Vignais and A. Baron-Van Evercooren*. U134 INSERM, Hôpital de la Salpêtrière, 75651 Paris cédex 13, France.

Several studies have provided evidence for the expression of the PDGF α-receptor (PDGF-αR) on oligodendrocyte progenitors (O-2A). The effects mediated by this receptor are proliferation, migration and chemoattraction. High levels of PDGF-A chain transcripts are detected in neurones of the embryonic and adult mouse central nervous system (CNS), suggesting that neurones may direct proliferation, targeting and finally differentiation of the oligodendrocyte progenitors prior to myelination. In this study, we analysed the expression of the PDGF-αR during the postnatal (P) development of the mouse CNS by in situ hybridization and immunohistochemistry on brain and spinal cord tissue sections. Between P1 and P15, PDGF-αR (transcripts and protein) are preferentially expressed by O-2A progenitors and pre-myelinating oligodendrocytes as previously reported. At P15, these cells with an O4-phenotype are in contact with PDGF-αR positive neurites in the cerebral cortex. At P21, this expression spreads throughout the brain and spinal cord and correlates well with the peak of myelination occurring at this time. In the mature CNS, PDGF-αR mRNA and protein are not detected in oligodendrocytes but are found in very few glial progenitors located in the cerebral cortex or the spinal cord white matter. Neural progenitors located in the subventricular zone and the ependymal/subependymal layer of the olfactory ventricle are also PDGF-αR positive. The most striking observation in our study is that mature neurones strongly express the PDGF-αR. In the forebrain, PDGF-αR positive neurones are noted in the mitral layer of the olfactory bulb, prefrontal and entorhinal cortex. In the cerebellum, Purkinje cells and deep nuclei neurones highly express PDGF-αR, as well as different brainstem nuclei, e.g, the facial and vestibular nuclei. In the spinal cord, neurones of the dorsal horn and motor neurones of the ventral horn are also labelled. This neuronal expression is first observed around P7 and extends to restricted central neuronal populations. The present data demonstrate a developmentally regulated sequential expression of the PDGF-αR early in glia and later in mature central neurones, suggesting a more extensive role for this receptor than previously described.

697.13

RAG-1-DEPENDENT IMMUNOGLOBULIN-LIKE IMMUNOREACTIVITY IN THE PERINATAL MURINE BRAIN.
J.A.Weiner* and J.J.M.Chun. Dept. of Pharmacology and Program in Neurosciences, Sch. of Med., Univ. of California, San Diego, La Jolla, CA 92093-0636.

Several reports have demonstrated immunostaining of cat, mouse and rat brains using species-specific anti-immunoglobulin-gamma (IgG) antisera. However, the identity of the immunostained molecule remains uncertain. Transcripts of the recombination-activating gene-1 (RAG-1), which encode one component of the putative V(D)J recombinase required for immunoglobulin gene rearrangements, have also been detected in the murine central nervous system. This finding suggests that some form of DNA rearrangement may occur there. Since IgG synthesis requires V(D)J recombination through RAG-1 expression, we have examined mice lacking the RAG-1 gene to determine whether the previously observed immunoglobulin-like immunoreactivity is RAG-1-dependent. We have employed three different antisera in this study, two of which recognize both heavy and light chains of mouse IgG (one containing whole antibodies and one consisting of $F(ab')_2$ fragments only), and one specific for the γ heavy chain. With all three antisera, we have observed immunostaining of the marginal zone and subplate of normal perinatal mice (E16-P1) similar to that previously reported. However, no immunostaining was apparent in the brains of mice from two independently derived and comparably aged RAG-1 -/- strains using any of the antisera, despite normal staining for known neuronal marker proteins. There are two possible interpretations of these results. Neurons in the immunostained areas may express an immunoglobulin-like molecule in a RAG-1-dependent manner, which is detected by anti-IgG antisera. Alternatively, neurons in the immunostained areas may bind or internalize serum IgG, which is absent in the RAG-1 -/- mice. We are presently investigating these possibilities. [We thank Drs. E. Spanopoulou and D. Baltimore for their RAG-1 -/- line. Supported by the Klingenstein Fund and NSF Graduate Fellowship.]

698.1

MORPHOMETRIC ANALYSIS OF CULTURED RAT BRAINSTEM MOTONEURONS: INFLUENCES OF GLIAL CELLS AND MUSLES. J.P. Ternaux*, P. Portalier and M.C. Chamoin. UPR 9041 CNRS, 280 Bd Sainte-Marguerite, 13009 Marseille, France.

Rat brainstems were sampled at day embryonic 15 to 18 and motoneurons were purified using a Nycodenz gradiant. Acetylcholine (ACh) content in the motoneurone fraction was 14 times higher than that detected in the glial and interneurones fraction. Purification of motoneurons (95 to 98 %) was further demonstrated using antibodies raised respectively against : ACh, choline acetyltransferase, Islet I and low affinity NGF receptors. Purified motoneurons were cultured in defined medium on glass coverslips coated with poly-L-lysine and laminin, with or without inserts containing either glial cells and/or myoblasts from tongue muscle. Growing motoneurons were observed during 7 days in culture and photographed. Morphometric parameters of 30 motoneurons selected at random were determined in each case, using a specific software. Survival of motoneurons was significantly increased in presence of glial cells and/or myoblasts inserts and total length of neuritic processes was significantly enhanced, with a marked effect on axons. No change was observed about the number of primary neurites but branching was significantly higher in the presence of a muscle insert. This effect disappeared when motoneurones were cultured in the presence of both glial cells and muscle inserts, suggesting a permissive influence of glial soluble factors on neuritic motoneuron branching.

698.2

DENERVATION-INDUCED DEATH OF TERMINAL SCHWANN CELLS IS CORRELATED WITH POOR REINNERVATION OF NEONATAL MUSCLE. W.J. Thompson* and J.T. Trachtenberg, Department of Zoology, The University of Texas, Austin, Texas 78712.

Denervation of neonatal rat muscles leads to apoptosis of terminal Schwann cells (Trachtenberg, this meeting). This contrasts with the situation in adult muscles where denervation leads to growth of Schwann cell processes from each endplate, processes that appear to be important in guiding axons during muscle reinnervation. In contrast to the adult, reinnervation of neonatal muscle is reported to be deficient, and the time period of this deficiency is roughly the time period of susceptibility of neonatal Schwann cells to axotomy-induced death. In attempt to test the importance of terminal Schwann cells for muscle reinnervation, we created nerve lesions in neonatal animals that led to return of regenerating axons either before or after the Schwann cells had died. Following crush of the nerve to soleus at its entry into the muscle, axons regenerated prior to the disappearance of many terminal Schwann cells. In these cases, the muscle was well reinnervated and the endplate band across the muscle was not much altered from normal (axons formed endplates in association with one or more terminal Schwann cells). However, in cases where nerve lesions were made at a greater distance from the muscle, regenerating axons arrived in the muscle after most terminal Schwann cells had died. In these muscles, many fewer fibers were successfully reinnervated, and the pattern of axon growth was abnormal. Axons generally grew through the old endoneurial tubes in the old endplate zone without branching. What few nerve terminal branches were present were associated with a few Schwann cells with long, extended processes. Thus, some Schwann cells are capable of migrating from the intramuscular nerves together with the regenerating axons; however, even these junctions were deficient in their Schwann cell composition. These results suggest that Schwann cell death may contribute to the poor reinnervation of neonatal muscles. These results also suggest that the persistent Schwann cells at adult junctions are important in reinnervation.

698.3

APOPTOSIS OF SCHWANN CELLS AT THE DEVELOPING RAT NEUROMUSCULAR JUNCTION IS REGULATED BY MOTOR NEURONS J.T. Trachtenberg* and W.J. Thompson, Department of Zoology, The University of Texas, Austin, Texas 78712.

Recent research from this lab has suggested that Schwann cells play critical roles in the processes of reinnervation and terminal sprouting in skeletal muscles Terminal Schwann cells overlying denervated endplates sprout elaborate processes which guide regenerating motor neurons and motor nerve terminal sprouts. In light of reports which suggest that reinnervation and terminal sprouting are deficient in neonates. I have examined the effects of muscle denervation by sciatic nerve resection on neonatal Schwann cells. Within 3 - 6 days of muscle denervation in rat pups aged P0-P14, 100% of terminal Schwann cells covering denervated endplates disappear. During the third postnatal week, the percentage of denervated endplates lacking terminal Schwann cells decreases, with no loss occurring after denervation on P25 or later. Use of end-labeling techniques employing terminal transferase and modified nucleotide substrates shows that the DNA of these cells fragments as they disappear. DNA fragmentation suggests that apoptotic death underlies Schwann cell disappearance. In muscles paralyzed with botulinum toxin, terminal Schwann cells remain over quiescent endplates, suggesting that Schwann cell death is regulated by neuronal rather than muscle derived trophic factor(s). Additionally, following botulinum toxin induced paralysis, terminal Schwann cells sprout processes which are associated with, but advancing in front of, nerve terminal sprouts. The results of these experiment suggest that 1) neuronal derived trophic factors regulate Schwann cell apoptosis at developing endplates, and 2) Schwann cell apoptosis following axonal degeneration, rather than functional deficiencies in neonatal Schwann cells, may underlie the deficiencies in reinnervation and sprouting in denervated neonatal muscle (Thompson, this meeting). Schwann cell apoptosis occurs at low levels in control, innervated muscles, suggesting that Schwann cell apoptosis may play a role in normal endplate development. Current efforts are aimed at identifying trophic factors which may rescue neonatal Schwann cells from death following denervation.

698.4

INFLUENCE OF THE AXON ON PRESYNAPTIC REGENERATION BY ADULT SENSORY NEURONS IN VITRO. M. Nachman-Clewner[1] and E. Townes-Anderson[2]*. [1]Cornell Univ. Med. Coll., New York, NY 10021, and [2]Dept. of Neurosciences, Univ. of Med. and Dentist. of NJ, Newark, NJ 07103.

Regeneration of neurites and presynaptic varicosities by cultured rod photoreceptors from the adult tiger salamander retina has recently been demonstrated. Quantitative variability in this regenerative response prompted us to investigate whether axonal lesion due to retinal disruption might promote process outgrowth and varicosity formation by these neurons. Cells were maintained *in vitro* as described (MacLeish and Townes-Anderson, 1988). Using Nomarski optics and time-lapse video microscopy, pairs of acutely isolated rod photoreceptors, in which one cell had lost and the other retained its original axon terminal, were selected and followed for 24h to 48h in culture. Cells were analyzed morphologically and immunocytochemically, utilizing antibodies to synaptic vesicle protein 2 (SV2) or synaptophysin (p38) to identify presynaptic sites, and to rod-specific opsin (4D2) to distinguish rod photoreceptors. Surprisingly, rod cells with an intact axon regenerated, on average, twice as many processes and five times as many presynaptic varicosities per cell as axotomized neurons. Axon retraction and elaboration of an axonal lamellipodium occurred by 24h *in vitro* in 75% of rod cells with a terminal upon plating, and varicosity bearing neurites developed from lamellipodial filopodia by 48h. Synaptic vesicle redistribution from terminal to lamellipodium to varicosities closely parallelled structural reorganization of the axon. By comparison, cells whose axon was lesioned exhibited virtually no synaptic vesicle immunoreactivity during early regenerative stages. As evidence for *de novo* protein synthesis, neurons were examined for colocalization of synaptic proteins with rab 6, a Golgi marker, in 0.5 µm optical sections by confocal microscopy. Initial formation of vesicle-filled varicosities preceded SV2/rab 6 colocalization. SV2 staining did not appear in the Golgi of cells with or without an axon until 2d to 7d *in vitro*. In contrast, rab 6 colocalized with opsin, a protein constitutively expressed throughout regeneration, even at 2h *in vitro*. Collectively, the data point to a positive role for the axon and suggest that SV protein synthesis is not a prerequisite for presynaptic regeneration. We propose a model in which regenerating neurons reutilize preexisting axonal components to expedite synaptic renewal as part of an early response to denervation. Supported by NIH Grant EY06135.

698.5

A RETINOTOPIC ANALYSIS OF THE DYNAMICS OF REGENERATING ARBORS IN LIVING ADULT GOLDFISH F.A.Johnson* and R.L. Meyer Dev. Biol. Center, University of California, Irvine, Irvine CA 92717

The formation of correct connections is crucial to the proper functioning of the nervous system. In the retinotectal system in goldfish, the initial projection is only roughly retinotopic and is progressively refined during regeneration such that ectopic synapses are eliminated in favor of retinotopic ones. This process is thought to depend on activity. In order to study the dynamics of this restructuring we labelled axons from a small area of retina and observed the arbors in living fish. Retinal arbors were labelled by a small injection of a fluorescent lipophilic neuronal tracer, DiI, into the retina of goldfish during the period of activity-dependent refinement, thirty to sixty days after an optic nerve crush. Five to seven days later, the tectum was exposed and the labelled arbors were observed with a cooled CCD camera. Images of the arbors were collected over five to eight hours to determine if the arbors had elongated or retracted in that time. Some regenerating and all normal arbors were stable in this time period, indicating that visualizing the arbors does not damage them. Many of the regenerating arbors observed exhibited some form of plasticity. Regenerating axon endings in tectal areas that had many other labelled endings, i.e. axons from cells in the same small area of retina, could be seen to grow or retract by small amounts- 10-20 µm. In contrast large retractions were observed when axons were separated from their retinotopic cohorts. Branches became filled with many large varicosities over the course of a few hours, then retracted 20-100 µm. In some cases axons seem to lose more than one branch in the same area. This suggests that axons can eliminate large portions of an arbor in a short time, and this may be more likely to occur in retinotopically inappropriate areas.

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698.6

STUDIES OF PHOTORECEPTOR AXONS REGENERATING *IN VITRO* FROM *DROSOPHILA* EYE DISCS. Chinglu Li*, X-J. Sun and L.A. Meinertzhagen. Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

Cells in the developing nervous system exhibit various signs of plasticity. We have used a primary culture system to study the development of the adult visual system in *Drosophila*. We have previously reported neurite extension from photoreceptors and endocrine regulation of eye disc differentiation *in vitro*. Here we report that newly differentiated photoreceptors in early pupae exhibit considerable plasticity, insofar as most extending neurites observed in culture have regenerated from severed photoreceptor axons. We used eye-imaginal discs from flies 5h after pupariation (P+5) in our cultures. Eye discs were cut into small fragments and dissociated by light enzymatic treatment with 0.05% trypsin for 15 mins. This partially dissociated the fragments, exposing the stumps of severed photoreceptor axons. These stumps initially floated in the culture medium. Their cell bodies were located in the disc fragment proper that adhered to the culture vessel. A few hours after culture initiation, the stumps attached to the substrate and started to elongate. Growth cones were frequently seen at the terminals of these regenerating axons. Punctate immunoreactivity to synaptotagmin, a synaptic vesicle protein, was detected along the neurites. Although the expression of synaptotagmin did not require ecdysone, the presence of this hormone caused more immunoreactive spots to appear along the length of the neurite. Growth cones were often foci of synaptotagmin immunoreactivity. Synapse formation occurs after photoreceptor axon growth cones collapse, *in vivo*. *In vitro*, the presence of synaptotagmin at growth cones implies the formation of vesicles. This suggests the presence of transmitter at growth cones, and immunoreactivity to histamine (HA, the photoreceptor transmitter) is indeed detected at the growth cone. It is possible that HA is released from growth cones and autoregulates neurite outgrowth. The effects of HA on neurite outgrowth will be presented. We also studied the ultrastructure of photoreceptor neurites in 5-d co-cultures of eye-disc fragments and optic lobe cells from early pupae. Junctions with a widened intercellular cleft, with synaptic vesicles, but lacking clear presynaptic ribbons, differentiate in the cultures. These observations suggest that severed photoreceptors are not only able to regenerate, but may also release transmitter *in vitro*. Supported by NIH grant (EY-03592).

698.7

SCHWANN CELL-COATED CARBON FILAMENTS AS STIMULATORS OF CNS AXONAL REGROWTH. S. Sayers*, T. Khan, K. Klein, G. DeVries, L. Liu. Rehab. R&D Center, Hines VA Hospital, Hines, IL 60141 and Dept. of Biochemistry, Medical College of Virginia, MCV Station Box 614, Richmond, VA 23298.

Regrowth of axons in the CNS occurs to a limited extent due to the nature of the environment. We have previously utilized small diameter (5μm) carbon filaments (Amoco Thornel ™) as an adjuvant to stimulate direct regrowth of transected axons in the CNS. We co-cultured Schwann cells (SC) with carbon filaments in the absence and presence of 14 day old rat fetal spinal cord tissue. We utilized a Human cell line (NF-1T) derived from a malignant soft tissue sarcoma from a patient diagnosed with neurofibromatosis. These cells showed the expected immunoreactivity to antigens known to be present on SC. In culture, these cells demonstrated the typical spindle spaced bipolar morphology and aligned themselves in fascicles.

Co-culture of NF-1T cells with dorsal root ganglion (DRG) neurites demonstrated that the NF-1T cells preferred the neuritic surface rather than the surface of other NF-1T cells. The ability of NF-1T cells to promote axonal outgrowth *in vitro*, together with the expression of markers known to be associated with SC, made them promising candidates for interacting with carbon filaments to further promote CNS axonal regrowth.

Scanning electron microscopy revealed that the NF-1T cells interacted with the surface of the carbon filaments aligning themselves with their long axis parallel to the long axis of the carbon filaments. The surface of the NF-1T cells was covered with numerous filopodia which appeared to aid in the stabilization of these cells on the carbon filaments. The presence of SC in the co-culture induced extensive neuritic outgrowth from the explants. Experiments to define the optimal ratio of NF-1T cells to carbon filaments are in progress. These experiments are designed to manipulate the NF-1T-carbon filament culture conditions so that the majority of the surface of the carbon filaments will be completely covered with NF-1T cells. In turn, these SC coated filaments should act as an effective stimulator of axonal regrowth *in vivo*.
Supported by NS15408 (GHDV) and Rehabilitation R&D Service, DVA.

698.8

RE-EVALUATION OF THE GROWTH PERMISSIVE SUBSTRATE PROPERTIES OF GOLDFISH OPTIC NERVE MYELIN

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Success of axonal regeneration in the goldfish optic nerve was previously found to correlate with the growth permissive substrate properties of fish CNS myelin. To determine if fish optic nerve myelin carries neurite growth inhibiting activities which would be neutralized by monoclonal antibody (Mab) IN-1 against mammalian neurite growth inhibitors (NI) (Caroni and Schwab, 1988), three sets of experiments with established methods were performed. (i) When fish optic nerve myelin was used as a substrate, both fish retinal ganglion cell (RGC) and rat DRG axons extended to considerable density and length. The presence of Mab IN-1 did not improve this growth, but unexpectedly Mabs O4 and O1 employed as "controls" reduced fish but not DRG axon outgrowth significantly. (ii) Axon growth on fish optic nerve myelin was quantified with IN-1 present in the medium and in assays in which myelin was preincubated with IN-1. In both, IN-1 had no effect on axon number or length. However, the same experiments with rat CNS myelin resulted in a marked rise of axon numbers and length with IN-1 as opposed to untreated myelin which hardly allowed any growth. (iii) Liposomes with incorporated CHAPS-extracted fish optic nerve myelin proteins were added to fish and DRG growth cones. One fraction was preincubated with IN-1 before use. 77% of fish and 89% of rat DRG axons continued to grow when contacted by the liposomes, and 80% of fish axons kept growing with IN-1 treated liposomes. Liposomes with CHAPS-extracted bovine CNS myelin proteins, however, induced collapse of 73% fish growth cones.

Thus, fish RGCs are sensitive to mammalian NI, but mammalian-like NI activities, which would be neutralized by IN-1, were not detectable in fish optic nerve myelin.
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698.9

DEVELOPMENTAL CONTROL OF RETINAL BIPOLAR CELL ARBORIZATION AND STRATIFICATION FOLLOWING INDUCED RETINAL ENLARGEMENT. J.C. Crowley, M. Xiong, B.D. Rubin, and B.L. Finlay*. Developmental Neuroscience Group, Cornell Univ., Ithaca, NY 14853.

Connectivity in the retina is established by either general or cell specific rules. The maintenance of correct dendritic and axonal arbor size plays a critical role in the maintenance of convergence ratios within the retina, which are responsible for the retina's balance between acuity and sensitivity. Previous work in this lab has described the relevant environmental factors that control the development of retinal ganglion cell arbors to be retinal ganglion cell density and the density of presynaptic input available to the cell. We manipulated retinal cell density in an effort to determine the effect of cell density on the production of bipolar axonal and dendritic arbors.

This study used visual form deprivation to experimentally enlarge the eye in the chick. Visual form deprivation induces ocular enlargement, producing a larger than normal retinal area and a reduction in RGC density of 20-30%. Ocular enlargement produced bipolar cells whose somas are 11.8% larger, whose axonal arbors are 36.8% larger, and whose axonal process had fewer stratifications. The number of IPL stratifications was also found to be inversely related to the total arbor size.

Center - periphery effects were also observed. Peripheral axonal arbor size increased 20.0%, an effect that was strongest in the experimental retina. The total length of the bipolar's dendritic process was also increased in the periphery, and a positive correlation was found between OPL and IPL arbor diameter.

These results are consistent with the results found previously for the RGC population of the chick; in both cases IPL arbors increase their coverage to fill the space made available by the retinal enlargement. The relationship between arbor size and number of stratifications suggests that in eye enlargement, growth is stimulated to such an extent that a physiological limit for either growth rate or cell size is reached and retinal coverage is conserved at the expense of IPL stratifications. In this way multistrata processing is subordinated to the maintenance of processing capability.
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698.10

DEVELOPMENTAL CONTROL OF DOPAMINERGIC AMACRINE CELL ARBORIZATION FOLLOWING PARTIAL OPTIC NERVE SECTION. M. Xiong*, J. C. Crowley, D. Troilo, M. Kelly, and B.L. Finlay, Developmental Neuroscience Group, Cornell Univ., Ithaca, NY 14853.

Regulation of survival and connectivity of widely branching, diffusely connected interneurons is an interesting and special case of afferent-target matching in the nervous system. Previously, visual form deprivation and optic nerve section were employed to study factors controlling arborization of retinal ganglion cells (RGC). Visual form deprivation induces ocular enlargement and retinal expansion. Partial optic nerve section causes degeneration of a portion of RGCs. We have reported that the growth of RGC arbor is limited in low RGC density region and branch density is increased, indicating a role of cell density and presynaptic availability in shaping RGC dendritic arbors. We adopted the same experimental paradigm to study the dopaminergic amacrine cell. Teakle et al (1993) have found that the size of dopaminergic arbors grows proportionately in expanded retinas to maintain their coverage. In this study, we examine arborization of the dopaminergic amacrine cell following partial optic nerve section.

Partial optic nerve section was performed in newly hatched chicks. Tyrosine hydroxylase immunohistochemistry was conducted on retinal wholemounts in four week old chicks. In RGC depleted retinas, cell density of dopaminergic cells remains constant and has a central to peripheral difference as in control retinas, that is, cell density becomes less dense towards the periphery. Soma size and dendritic arbor area are not changed. In contrast, mean branch length is increased, which corresponds to a decrease in branch number in higher order branches and no change in total arbor length.

We conclude that dopaminergic amacrine cells do not die or change their neurotransmitter complement following early RGC depletion. Also, a change in RGC contribution to the IPL to fewer, denser RGC arbors causes a reduction of the dopaminergic cell branching while coverage of retinal area is maintained.
(Supported by R01-NS 19245 & T32-MH19389)

698.11

ULTRASTRUCTURAL CHARACTERISTICS OF SEPTOHIPPOCAMPAL AXONS SURVIVING TRANSECTION G. M. Peterson*. Dept Anatomy & Cell Biology, East Carolina Univ Sch Med, Greenville, NC 27858.

We have previously shown that some of the cell bodies of septohippocampal neurons survive for weeks to months following transection of their axons. Additionally, we have shown that some of their axons also survive for weeks to months without signs of retraction. The purpose of the present study was to examine the ultrastructure of these putative surviving, axotomized axons. Bilateral aspirative lesions of the fimbria-fornix were made mid-way between the medial septum (MS) and septal pole of the hippocampus in adult female Sprague-Dawley rats. One week later an iontophoretic injection of PHA-L was placed into the MS. Following an additional week, the animals were killed and brain sections were cut on a vibratome. Sections through the septal area and the region of the lesion were immunocytochemically stained for PHA-L using DAB as the chromogen. Hippocampal sections were histochemically stained for AChE as an indicator of the extent of septal cholinergic denervation. PHA-L stained sections were osmicated, stained with uranyl acetate and phosphotungstic acid, flat embedded in Epon, and photographed at the light microscopic level. Regions containing labeled axons were then selected, trimmed, thin sectioned, and mounted on mesh grids. Sections were examined in a JEOL 1200EX electron microscope. Labeled axons showed the granular appearance characteristic of DAB and were unmyelinated. Many of the surrounding unlabeled axons were myelinated. Due to the granular DAB labeling, it was difficult to identify the smaller axonal constituents, but mitochondria were present, often in pairs. Labeled axons had a diameter of 0.7 to 1 μm and were unbranched. Growth cones were not observed. Neither the labeled nor the unlabeled axons showed obvious signs of degeneration. It is concluded that a portion of septohippocampal axons survive at least two weeks after axotomy and that their ultrastructure is relatively normal.

698.12

RE-EXPRESSION OF THE DEVELOPMENTALLY REGULATED PROTEIN FAC1 IN DENTATE GYRUS FOLLOWING ENTORHINAL CORTEX LESION S. D. Styren*, R. Bowser, G. C. Styren, M. E. O'Malley and S.T. DeKosky Depts. of Psychiatry, Neuropathology, Neurology, and Neurobiology Western Psychiatric Inst. & Clinic and Univ. of Pittsburgh Sch. of Med. Pittsburgh, PA 15213

FAC1 is a novel developmentally regulated protein present in neuronal cytoplasm during brain development. In mature animals cellular localization shifts to neuronal nuclei and cytoplasmic expression is lost. FAC1 has been shown to be re-expressed in neurodegenerative disease and is localized to both neuronal cytoplasm and a subset of neuritic plaques in Alzheimer's disease. To further analyze the role of this protein during neuronal injury, we denervated the rat hippocampal dentate gyrus molecular layer (ML) by entorhinal cortex (ERC) lesion and assessed FAC1 distribution with two monoclonal antibodies directed against distinct FAC1 epitopes. FAC1 staining was absent from the ML in unlesioned animals. By 2 days post ERC lesion there was intense FAC1 immunoreactivity spanning the denervated outer ML; the inner ML remained unstained. We examined additional animals at 6, 15 and 30 days post lesion, during which time commissural and associational (C/A) afferents from inner ML are known to sprout partway into the denervated zone. FAC1 staining continued to be localized to the outer ML at each time point, but appeared to recede from the interface of inner ML trans-laminar expansion. We previously demonstrated rapid re-expression of the embryonic form of neural cell adhesion molecule (eN-CAM) in the denervated outer two thirds of the molecular layer following ERC lesion. FAC1 expression, following ERC lesion, is both spatially and temporally analogous to eN-CAM expression and closely follows the time course of reactive synaptogenesis and axonal sprouting. Re-expression of FAC1 in denervated dentate ML suggests a role for this developmental protein in CNS response to injury.

698.13

NEW AXONAL GROWTH IN THE DEAFFERENTATED MEDIAL HABENULA OF ADULT RATS. J.A Wilson* and M.D. Kawaja. Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, CANADA K7L 3N6.

In the normal adult rat brain, the posterior septal nuclei project ipsilaterally through the stria medullari (*sm*) to provide a dense innervation of calretinin-immunoreactive axons to the cholinergic neurons of the medial habenula (MHb). In this study, we investigated the plasticity of the septo-habenular projection, one and four weeks after unilateral MHb deafferentation. At one week, there is a complete loss of calretinin-positive fibers throughout the entire rostro-caudal extent of the ipsilateral MHb; the contralateral MHb remains unchanged from controls. At four weeks, however, calretinin-positive axons and clusters of terminals are again evident in the ipsilateral MHb, yet confined to the caudal-most portion of the nucleus. Immunostaining for neurofilament-200 and GAP-43 also reveals a higher density of reactive fibers in the caudal, as compared to rostral, aspect of the ipsilateral MHb. Following injections of biotinylated dextran amine into the ipsilateral MHb at 4 weeks after unilateral deafferentation, labelled axons are evident in the habenular commissure and contralateral *sm*, as well as labelled somata in the contralateral posterior septum. In those animals with bilateral lesions of the *sm*, there are no calretinin-positive fibers found within either MHb after 4 weeks. These data reveal a remarkable degree of plasticity among septal inputs to the adult MHb following unilateral deafferentation. We propose that the new calretinin-positive fibers found in the deafferentated MHb arise from neurons in the contralateral septum which project through the intact *sm* and into the habenular commissure. (Supported by Queen's Univ.)

698.14

STRIATAL TARGET NEURONS INCREASE AXONAL BRANCHING OF DOPAMINERGIC MESENCEPHALIC NEURONS IN CULTURE. M.Manier[1], C. Chatellard[1], P. Mouchel[1], J.P. Herman[2] and C. Feuerstein[1]*. 1. INSERM-LAPSEN U318, 38043 Grenoble; 2. UMR CNRS 9941, 13916 Marseille, France.

Target striatal cells were shown to increase some biochemical aspects of the development of dopaminergic (DA) mesencephalic neurons in culture. In the present study, we attempted to define which aspects of DA neurons development is typically influenced by striatal neurons. Embryonic DA neurons of rat mesencephalon were cocultured in presence of neurons derived from either target striatal or non target cerebellar tissue. After 4 days of dissociated culture in a chemically defined, serum-free medium, two aspects of the development of DA neurons were studied: (1) morphological , the morphometric analysis of DA neurons outgrowth after tyrosine hydroxylase (TH) immunohistochemistry (2) biochemical, the quantitative analysis of intracellular TH contents after Western blot analysis and after densitometry of TH immunolabeling in individual cells.

The total length and particularly the complexity of TH+ neurites (number of branching points) were dramatically increased when DA neurons were cocultured in presence of striatal cells but not cerebellar ones. To define what type of neurites was concerned, TH immunofluorescence was coupled with that of specific markers of either dendritic (MAP2) or axonal (phosphorylated neurofilament) compartment. It was thus shown that the presence of striatal neurons induced highly branched axones, without changes of the dendritic arborization. Due to the culture conditions (serum-free medium), the target striatal neurons themselves and not striatal glia were accountable for this peculiar effect on the axonal outgrowth of DA mesencephalic neurons. Conversely, the increase in intracellular TH contents in DA neurons observed in parallel, appeared to be influenced to the same extent by the presence of either target or non target neurons.

698.15

SYNAPTIC REORGANIZATION IN RAT HIPPOCAMPAL FORMATION FOLLOWING DORSAL SEPTOHIPPOCAMPAL LESION. H.Hong, D.Kim J. Lee, T.Jung and S. Inagaki* Dept. of Anatomy, Sch. of Med. Ulsan Univ. Seoul, Korea; *Lab. Sciences, Sch. of Allied Health Sci. Osaka Univ. Faculty of Med. Osaka, Japan.

Synaptic reorganization in rat hippocampal formation following dorsal septohippocampal pathway lesion were determined by employing immunohistochemical method with the antibody specific to growth associated protein(GAP-43) . Within the 1st week, there was a significant decrease in the GAP-43 immunoreactivity(IR) in the supragranular layer of dentate gyrus and stratum lacunosum moleculare of the CA1 field. Between 2 and 3 weeks after lesioning, levels of the GAP-43 IR were found to increase markedly in the inner molecular layer of the dentate gyrus, coincident with the time at which commissural-associational fibers sprout axon collaterals into dendritic portions denervted by the lesion. In the stratum lacunosum moleculare, GAP-43 IR was recovered at 1 and 2 weeks later, presumably due to the sprouting of other inputs. GAP-43 levels in the ipsilateral supragranular layer after the lesion of septohippocampal pathway did not return for 12 weeks, suggesting that ipsilateral supragrnular layer did not receive terminal fibers via any other routes after dorsal septohippocampal path lesion. In contrast to the ipsilateral side, level of GAP-43 IR in the supragranular layer contralateral to the lesion increased during times of terminal axon sprouting, interestingly.

698.16

VASOPRESSIN mRNA LEVELS AND CYTOCHROME OXIDASE ACTIVITY IN THE HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM DURING AXONAL SPROUTING: EFFECTS OF HYPONATREMIA. C.W. Moffett*, J.P. Herman[1] and C.M. Paden. Dept. of Biology and WAMI Medical Program, Montana State Univ., Bozeman, MT 59717 and [1]Dept. of Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington KY 40536.

We have previously shown that uninjured magnocellular neurons on one side of the rat hypothalamus will grow significant numbers of new axon terminals in the neural lobe (NL) following destruction of the contralateral projection to the NL from the paraventricular and supraoptic (SO) nuclei (Watt and Paden, *Exp. Neurol.* 111, 9, '91). This robust collateral sprouting response is accompanied by an increase in urine osmolality, suggesting that magnocellular neurons are hyperactive while axonal sprouting is occurring. To test this hypothesis we are measuring (1) vasopressin mRNA levels in the sprouting SO by *in situ* hybridization and (2) cytochrome oxidase activity in both the sprouting SO and NL terminal field by histochemistry. In order to determine if alterations in neuronal activity will affect the sprouting response, we are also investigating the effects of reducing neurosecretory activity through chronic hyponatremia (Verbalis and Drutarosky, *Kidney Int.* 34, 351, '88). Results obtained to date indicate that vasopressin mRNA levels in the intact (sprouting) SO are significantly increased at 7 days after a contralateral hypothalamic lesion. This increase is blocked by prior induction of chronic hyponatremia, which also results in a reduction of cytochrome oxidase activity in the NL. These initial findings support the hypothesis that an increase in neurosecretory activity occurs during the initial period of axonal sprouting. Analysis of sections from animals sacrificed during the peak of the sprouting response at 28 days post-lesion is in progress, and should aid in determining the relationship between neuronal activity and collateral sprouting. Supported by NINDS NS32507 to CMP.

698.17

AXONAL SPROUTING IN THE HIPPOCAMPAL DENTATE GYRUS IN NCAM DEFICIENT MICE. I.M. Fugaccia and S.W. Scheff*. Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536.

The hippocampal dentate gyrus has been used extensively to study plasticity in the CNS. Following partial denervation of the granule cells, a morphological change occurs among the residual afferents innovating the dentate molecular layer. One aspect of this change is the growth of the commissural/associational (C/A) fibers following removal of the ipsilateral entorhinal cortex (EC). Neural cell adhesion molecules (NCAM) play a central role in the developing nervous system where they influence axonal growth, guidance and synapse formation. They have been implicated in peripheral nervous system regeneration. Little is known of their role in axonal sprouting in the adult CNS.

NCAM deficient mice generated by gene targeting were compared to normal mice for their ability to support reactive outgrowth of the C/A fibers. Mutant and normal mice were subjected to a unilateral removal of the EC and allowed to survive for 15 days following surgery. The hippocampus was then processed for light microscopy and stained for AChE histochemistry to assess changes in the septohippocampal afferents as well as with the Holmes fiber method to assess changes in the C/A fiber plexus.

Both normal and NCAM deficient mice displayed intensified AChE staining in the outer molecular layer indicative of septohippocampal sprouting. Analysis of the outgrowth of the C/A fibers revealed robust axonal growth and no significant difference between the two groups was observed. These results indicate that although NCAM may play an important role in brain plasticity when present, it is not necessary for all CNS self-repair. Supported by AG05144

698.18

RAPID INDUCTION OF AXONAL GROWTH AND F1/GAP43 PROTEIN IN THE HIPPOCAMPAL MOSSY FIBERS OF THE ADULT RAT. I. Cantallops, J. Moskal*[1] and A. Routtenberg. Cresap Neuroscience Laboratory, Northwestern University, Evanston, IL 60208; [1] Chicago Inst. Neurosurg., Chicago, IL 60614.

Granule cells in the adult rat hippocampus do not normally express axonal growth- and plasticity-associated protein F1/GAP43 (a.k.a. B-50 or neuromodulin). Hypersynchronous activity in limbic circuits and behavioral seizures caused by 10 mg/kg (sc) kainic acid (KA) are followed by induced expression of F1/GAP43 mRNA in granule cells. Subsequently, the axons of granule cells, the mossy fibers, show dramatic sprouting. We demonstrate here that this sprouting can be detected in temporal hippocampus within 2 days. By combining the study of F1/GAP43 immunoreactivity and mossy fiber sprouting in the same animal, adult axonal growth is linked to protein F1/GAP43 expression

F1/GAP43 mRNA expression in granule cells in the temporal, but not septal, hippocampus was induced beginning at 12 hr after KA treatment. This is preceeded by alteration in E-box transcription factor binding. Beginning 2 days after KA, mossy fiber sprouts were observed in the supragranular layer of the temporal, but not septal, hippocampus. An apparent increase in levels of protein F1/GAP43 immunoreactivity in the supragranular layer was observed at this same 2 day time point and ventral hippocampal location. F1/GAP43 protein levels and mossy fiber sprouting showed an increase up to 10 days after KA. Sprouting was at a maximum at 40 days, the longest time point studied.

The rapid onset of axonal growth in the adult rat is striking and occurs earlier than reported previously (2 days vs. 12 days). The time course of molecular events observed in this study is similar to that observed during axonal regeneration with one critical difference: granule cell axons do not appear to be damaged by kainate. (Supported by "La Caixa" Fellowship (Spain) to I.C. and MH25281-21 to A.R.).

698.19

SEIZURES INDUCE THE EXPRESSION OF TENASCIN-C mRNA AND PROTEIN BY NEURONAL AND GLIAL CELLS. J. Niquet, L. Ferhat, N. Chevassus, M. Khrestchatisky, Y. Ben-Ari and A. Represa*
Université René Descartes (Paris VI). INSERM U29, 123 Bd. Port Royal 75014 Paris (France).

Temporal lobe epilepsy is associated with neuronal death, gliosis and sprouting of mossy fibers (i.e. the axons of dentate granule cells) in the hippocampus of human and rats. This has been well analyzed in the model induced by kainate injection (see Represa et al. '95 J. Neurobiol. 26, 413 for a review).

In the present study we show that immunoreactivity and mRNA hybridization of tenascin-C (an extracellular matrix glycoprotein with repulsive properties) increase in the hippocampus of rats treated with kainate. The increase of tenascin-C was particularly striking in Ammon's horn, where tenascin-C probes and antibodies labelled reactive astrocytes. Tenascin mRNA expression was also induced in granule cells and protein immunoreactivity appeared in plasma membranes of their axons, the mossy fibers. Tenascin-C immunoreactivity remained unchanged in the molecular layer of epileptic rats.

It is interesting that increased tenascin-C immunoreactivity was observed within zones in which axonal regeneration does not occur (the CA3 area in kainate treated animals) whereas zones in which reactive synaptogenesis occurs (the molecular layer) were devoid of tenascin-C immunoreactivity. We may infer from these results that tenascin-C impedes the terminal sprouting of mossy fibers in CA3 of kainate-treated rats.

698.20

ROLE OF HIPPOCAMPAL COMMISSURE AND HILAR CELLS IN KAINIC ACID-INDUCED MOSSY FIBER SPROUTING D. L. Fairbrother, D. Terrian*, and G. M. Peterson. Dept Anatomy & Cell Biology, East Carolina Univ Sch Med, Greenville, NC 27858.

Aberrant sprouting of mossy fibers (MF), which are the axons of dentate gyrus granule cells, occurs in epilepsy and can be experimentally induced by intraventricular injections of kainic acid (KA). It has been reported that MF sprouting occurs more rapidly after bilateral KA than after unilateral KA. A possible explanation for this difference is that commissural afferents from the contralateral dentate gyrus sprout and compete with the ipsilateral mossy fibers, thereby retarding their sprouting. To investigate the role of the commissural afferents in mossy fiber sprouting, rats were divided into three treatment groups: unilateral KA, bilateral KA, and unilateral KA with severance of the hippocampal commissure. Animals from each group were killed at 2, 3, and 4 weeks after treatment and brain sections were prepared (Timm and Nissl) and analyzed to determine the extent of mossy fiber sprouting and cell loss within the hippocampus. Animals which received either bilateral KA or unilateral KA together with transection of the hippocampal commissure exhibited more rapid and dramatic mossy fiber sprouting than did the animals which received only unilateral KA. These data provide indirect support for the hypothesis that sprouting by commissural afferents retards simultaneously sprouting mossy fibers. Additionally, we found a positive correlation between the degree of mossy fiber sprouting and neuronal loss in the hippocampus. Correlation was found only with hilar cells and was highest in the most ventral region of the dentate gyrus. A possible interpretation of this result is that loss of hilar neurons is specifically involved in the induction of mossy fiber sprouting. Together, these two observations elucidate some of the factors contributing to mossy fiber sprouting.

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING VI

699.1

THE CONTINUOUS PRESENCE OF NERVE GROWTH FACTOR IS NOT NECESSARY TO PROMOTE NEURITE OUTGROWTH IN CHICK EMBRYONIC SENSORY GANGLIA. S. Mehta[1], L. Hsu[2]* and K. Y. Chen[1]. [1]Dept. of Chemistry, Rutgers Univ., New Brunswick, NJ 08906 and [2]Dept. of Biology, Seton Hall Univ., S. Orange, NJ 07079.

Previously, we have shown that a brief exposure to the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) for 30 min was sufficient to elicit intense neurite outgrowth in embryonic ganglia explants (Mehta et al., 1993, *J. Neurochem.*, 60:972-981). We now report that addition of 50 ng/ml of Nerve Growth Factor (NGF) to serum-free growth medium for 30 min was also capable of promoting neurite outgrowth from 10 day old embryonic sensory ganglia explants. Unlike treatment with TPA, NGF-stimulated neurite outgrowth was not correlated with an increase in the phosphorylation of the 66-kDa substrate of protein kinase C. While the combined treatment of ganglia explants with NGF and the PKC-inhibitor staurosporine for the entire 48 hrs blocked neurite outgrowth, a cotreatment of both reagents for the critical 30 min period did not inhibit process outgrowth. The suppression of NGF-stimulated but not dibutyryl cAMP-mediated neurite outgrowth by staurosporine suggests the involvement of PKC but not PKA in neurite outgrowth beyond the initial 30 min period.

699.2

A NOVEL NEURITE OUTGROWTH PROMOTING FACTOR SPECIFIC TO EMBRYONIC TELENCEPHALIC NEURONS H.Nishimune*,A.Uyeda,M.Nogawa,T.Taguchi+
Lab. of Mol. Biol.,Fac. of Eng.Sci., Osaka Univ., Toyonaka 560, Japan, + Osaka Nat'l. Res. Inst. AIST, Ikeda 563, Japan

A novel neurite outgrowth promoting factor(NPF) has been cloned from cDNA library of denervated chick muscle. Neurite promoting activity of the recombinant muscle-derived NPF(mNPF) was found to be specific to telencephalic neurons in dissociated primary culture of embryonic chick brain neurons. This activity coincides with that of a muscle-derived factor in our previous report. The size of mNPF mRNA is 3.7kB and the expression of mNPF in skeletal muscle was revealed to be up-regulated by denervation. The sizes of an expressed protein is 115kD. The deduced amino acid sequence of NPF indicates a highly hydrophilic property but no consensus sequence of trophic factors nor cell adhesion molecules exists. We further analyzed to search for any homologue in the telencephalon and obtained a clone(tNPF) with high homology with mNPF. The expression of tNPF peaks at around embryonic day 10 which is known to be a time just before synaptogenesis. The NPF does not exhibit high homology with previously known target-derived diffusible factors such as neurotrophic factors nor components of extracellular matrix such as laminin.

699.3

ANGIOTENSIN(3-8) [ANG IV], INHIBITS NEURITE OUT-GROWTH FROM CULTURED CHICK SYMPATHETIC GANGLIA. I. Moeller, [1]D.H.Small, [1]G.Reed, [2]J.W.Harding, S.Y.Chai and F.A.O.Mendelsohn*. University of Melbourne, Department of Medicine, Austin & Repatriation Medical Centre, Heidelberg 3084, [1]Department of Pathology, University of Melbourne, Parkville 3052, Victoria, Australia, [2]Department of VCAPP, Washington State University, Pullman, WA, U.S.A.

Angiotensin (3-8), [Ang IV], a metabolite of angiotensin II, (Ang II), has been associated with increasing memory retrieval and dilating pial arterioles.

Binding sites for Ang IV, which occur throughout the guinea pig, monkey and sheep brain, are abundantly localized in components of the autonomic nervous system:- the nucleus ambiguus, dorsal motor nucleus of the vagus and the rostral ventrolateral medulla, whilst in the sheep spinal cord, binding sites are present in the sympathetic preganglionic neurons.

In the present study, 1nM-100μM Ang IV, inhibited neurite outgrowth from cultured E11 chick prevertebral sympathetic ganglia, by 20-30%. The 10nM Ang IV effect was totally inhibited by 1μM of the Ang IV analogues, WSU 4042, Nle^1-Y-I-amide and Nle^1-AIV, but was unaffected by the Ang II antagonist [Sar^{1}Ile^8]Ang II, and the AT_1 and AT_2 receptor subtype antagonists, Losartan and PD 123319, respectively. Ang II (1nM-100μM), also inhibits neurite outgrowth in this system, but may be acting via the Ang IV binding site since its effect is reversed by the Ang IV analogues and not by the Ang II antagonists.

These results suggest that Ang IV may have trophic functions in the central nervous system, particularly in the autonomic system.

699.4

NEUROIMMUNOPHILIN LIGANDS ARE NEUROTROPHIC FOR PC12 CELLS AND SENSORY NEURONAL CULTURES. J. P. Steiner*[1,4], T. M. Dawson[4], M. A. Connolly[1], G. S. Hamilton[1], P. D. Suzdak[1] and S. H. Snyder[4].
Departments of Neurobiology and Medicinal Chemistry, Guilford Pharmaceuticals[1] and Departments of Neurology and Neuroscience[4], The Johns Hopkins University School of Medicine, Baltimore MD21224

The immunosuppressant drug FK506 acts by binding to receptor proteins called FKBPs, which in turn can bind to and regulate a calcium/calmodulin-dependent protein phosphatase, calcineurin, and intracellular calcium release channels, such as the ryanodine receptor and inositiol-1,4,5-trisphosphate receptor. Recently, we have demonstrated that the immunosuppressant drug FK506 promotes neurite outgrowth in PC12 cells and sensory ganglion cultures (Lyons et. al., Proc. Nat'l Acad. Sci. USA 91, 3191-3195 (1994)). Picomolar concentrations of FK506 enhance neurite outgrowth in both systems by increasing sensitivity to nerve growth factor. We have extended these studies to include the immunosuppressant drug rapamycin, which at low nanomolar concentrations, promotes neurite extension in PC12 cells and chick dorsal root ganglion cultures by increasing sensitivity to NGF. The cellular effects of rapamycin are mediated by interaction with FKBP-12 and the rapamycin and FKBP target protein (RAFT). These results suggest that the neurotrophic effects of the neuroimmunophilin ligands may be mediated by interactions with multiple signal transduction pathways.

699.5

ANTI-IDIOTYPIC ANTIBODIES TO GM1 ENHANCE GROWTH FACTOR STIMULATED NEURITE ELONGATION. M.J. Riggott* and W.D. Matthew. Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710

We have tested the ability of anti-idiotypic antibodies to GM1 (AIG Mab's) to stimulate neurite outgrowth in a bioassay using cultures of dorsal root ganglion (DRG) neurons. Bioassays were developed using adult DRG neurons cultured on poly-lysine in the presence and absence of growth factors. Three indices of neurite outgrowth were measured for each neuron: the length of the longest neurite, the number of primary neurites and the number of branch points. The DRG neurons were cultured in the presence of AIG4, AIG5, AIG20, control antibodies or NS1 conditioned media. To test the dependence on growth factors, these experiments were conducted in one of the following media preparations: a) no added growth factors; b) a suboptimal concentration of NGF; c an optimal concentration of NGF plus N2 (insulin, transferrin, progesterone, putrescene, selenite).

The AIG Mab's have the ability to promote neurite elongation under all conditions. However, elongation is most pronounced when DRG neurons are cultured in the presence of growth factors and either antibody AIG4 or AIG20. The growth factors have a negligible effect on neurite length in the absence of AIG Mab's. The number of primary neurites and the number of branch points were not affected in these assays. This suggests that the AIG Mab's may facilitate the activity of growth factor activated systems that regulate neurite elongation. This work was supported in part by a grant from the Paralyzed Veterans of America Spinal Cord Research Foundation.

699.6

DIFFERENTIAL EFFECTS OF MICROTUBULE INHIBITORS ON AXONAL BRANCHING AND ELONGATION OF CULTURED RAT HIPPOCAMPAL NEURONS. A. Aoyagi*, H. Saito and K. Abe. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo 113, Japan.

We have previously found that basic fibroblast growth factor (bFGF) specifically enhances neurite branching without affecting neurite elongation of cultured hippocampal neurons, while astrocyte-conditioned medium (ACM) promotes the axonal elongation but not branching. This finding suggests that the two morphological processes, i.e. neurite elongation and branching, are independently regulated by different mechanisms, and further investigations about the action of bFGF will help us to understand the machanisms involved in neuritogenesis and synaptogenesis of brain neurons. In the present study, to test the role of microtubules in the axonal branching and elongation, we compared the effects of microtubule inhibitors, taxol and colchicine, on the actions of bFGF and ACM. The dissociated hippocampal neurons from 18-day-old embryos of Wistar rats were plated on polylysine-coated culture dishes at a density of 2,500 cells/cm^2. After incubation in serum-containing medium for 24 hrs, the medium was changed to serum-free medium. The cells were cultured for further 24 hrs, and then neurons bearing processes were randomly selected and photographed before addition of drugs. The same cells were photographed 24 and 48 hrs after addition of drugs, and changes in morphological parameters were compared. Taxol (50 nM) alone did not affect the morphology of neurons cultured under control conditions, but significantly reduced the bFGF-stimulated axonal branching. The ACM-stimulated axonal elongation was not affected by the same concentration of taxol. Colchicine (10 nM) showed similar effects as taxol. These results suggest that microtubules play more important roles in the axonal branching than in the axonal elongation.

699.7

STEROID-ENHANCED NEURITE OUTGROWTH IN INSECT MOTOR NEURONS INVOLVES HIGHER-ORDER BRANCHING AND INCREASED GROWTH CONE COMPLEXITY. S.F. Matheson*, K. Della Croce and R.B. Levine. Div. of Neurobiol., Univ. of Arizona, Tucson, AZ 85721

Extensive remodelling of neuronal processes during metamorphosis is a prominent feature of nervous system development in the moth, Manduca sexta. Leg motor neurons (MNs), which are retained through metamorphosis, undergo severe dendritic regression at the end of larval life, then grow a new, complex dendritic arbor during metamorphosis. This restructuring is controlled by the steroid molting hormone, 20-hydroxyecdysone (20HE). Previous work in our laboratory has shown that leg MNs maintained in cell culture respond to 20HE exposure by growing more elaborate neuritic arbors. In order to define the cellular mechanisms underlying this morphological effect, we are comparing specific characteristics of neuritic branching patterns, and growth cone complexity and cytoskeletal structure, in leg MNs cultured with and without 20HE. Morphometric data suggest that 20HE induces a dramatic increase predominantly in higher-order neurite branching. Neuronal growth cones display typical complements of microtubules and filamentous actin: microtubules are concentrated in a central region and filamentous actin accumulates in the growth cone periphery, in numerous microspikes and small filopodia. Lamellipodia are almost never observed; large flattened areas are common but are heavily invested with microtubules. Growth cones are more highly branched in the presence of 20HE, and display significantly more microspikes and filopodia as revealed by fluorescent staining of actin filaments. These results suggest that 20HE induces increased morphological complexity in cultured leg MNs by enhancing higher-order branching, perhaps via an effect on growth cone complexity and motile activity. Supported by NIH grant NS28495.

699.8

cDNA CLONING AND EXPRESSION OF XEFILTIN, A NOVEL NEURONAL INTERMEDIATE FILAMENT PROTEIN IN *XENOPUS LAEVIS*. Y.ZHAO* and B.G.SZARO. Biol. Sci., SUNY-Albany, Albany, NY 12222.

During axonal development and regeneration, the expression and post-translational modification of neuronal intermediate filament (NIF) proteins are cell-type specific and highly regulated. The changing molecular composition of the neurofilaments may help to regulate axonal stability at different stages of growth and to influence the caliber of diverse types of axons. Consequently, it has been suggested that NIF composition also affects the capability of a neuron for growth and modification. Consistent with this idea, fish and amphibians have unique NIF proteins whose patterns of expression suggest they may confer on these animals a higher capacity for plasticity and regeneration. We report here the isolation of full length cDNA clones that encode a novel *Xenopus* NIF protein with a predicted molecular weight of 55.9 kD, which we have named xefiltin. Xefiltin shared high amino acid sequence identity both with goldfish gefiltin (overall: 66%, head: 59%, rod: 76%, tail: 30%) and with rat α-internexin (overall: 64%, head: 61%, rod: 71%, tail: 45%); however, its pattern of expression was unique. Unlike gefiltin, which is predominantly found in the retina, xefiltin mRNA was expressed throughout both the CNS and the PNS, as demonstrated in juvenile frogs by Northern blots and *in situ* hybridization. In preliminary analyses by Northern blot, xefiltin mRNA was detected later than that of *Xenopus* NF-L. This differs from α-internexin, which appears before NF-L during mammalian development. Further characterization of xefiltin expression during *Xenopus* neural development and optic nerve regeneration and alteration of its expression in the embryo should provide an additional focus for understanding how modifications of the neuronal cytoskeleton affect axonal growth and development. Supported by NINDS grant R29-NS30682.

699.9

DEVELOPMENT, SUBCELLULAR LOCALIZATION AND REGULATION OF ALPHA INTERNEXIN IN HIPPOCAMPAL NEURONS D. L. Benson* and J. L. Brown Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, New York 10029

The differential distribution and organization of cytoskeletal elements is the basis for many of the morphological and functional differences between axons and dendrites. Alpha internexin is a neuronal intermediate filament protein which is expressed at highest levels in the CNS during development, but continues to be expressed throughout adulthood. Immunocytochemical localization of α–internexin in cultured hippocampal neurons revealed the intermediate filament in all neurons and axons as well as dendrites. Coincident with the developmental time of dendritic maturation and synaptogenesis, α–internexin became concentrated in axons where long filaments could be labeled. Labeling in dendritic shafts was much fainter and fuzzier, and was also present within dendritic spines–some of which could be labeled simultaneously with antibodies against glutamate receptors 2/3. Labeled spines were not observed on neurons with a GABAergic morphology. In perfused tissue sections of adult rat hippocampus, α–internexin appeared to predominate in dendrites rather than axons. Electron microscopy revealed immunolabel within dendritic spines and associated with microtubules in dendritic shafts. Only a small population of axons was labeled. Following entorhinal cortex lesions α–internexin immunolabel increased in regions undergoing synaptogenesis. Although it remains to be determined whether the increase was pre- or postsynaptic, it appears that α–internexin expression is positively correlated with synaptogenesis and may also be involved in the generation or maintenance of dendritic spine structure. *Supported by NSF grant IBN-9419900.*

699.10

EVIDENCE FOR A NEURONAL MYOSIN LIGHT CHAIN KINASE: cDNA CLONING AND LOCALIZATION IN GOLDFISH. X.Jian*, B.G.Szaro and J.T.Schmidt. Dept. of Biol. Sci., SUNY-Albany, Albany, NY 12222.

The control of actin-myosin interaction, which has been implicated in growth cone motility and neurite outgrowth, is regulated in non-skeletal muscle cells by myosin light chain kinase (MLCK). Pharmacological reagents specific for MLCK alter neuritic outgrowth of cultured goldfish retinal ganglion cells (Jian et al., J.Neurobiol. 1994 25:1310), and an antiserum directed against chicken gizzard MLCK stains rat brain neurons and glia (Edelman et al., Mol. Brain Res. 1992 14:27). To establish further proof of the existence of MLCK in neurons, a cDNA probe derived from chicken fibroblast MLCK (CF-MLCK) was used to isolate cDNA clones from separate goldfish retinal and brain libraries. These clones encoded three isoforms that all shared highest sequence similarities with known smooth muscle and nonmuscle MLCK's from various avian and mammalian species. Partial sequences obtained from these clones were compared with that of CF-MLCK. Over the regions encoding the conserved catalytic and calmodulin regulatory domains of the kinase they all shared 78% identity at the nucleotide level with CF-MLCK. *In situ* hybridization experiments performed with a cRNA probe derived from one of the clones revealed specific hybridization with message in several regions of the goldfish brain, most abundantly in neurons of the periventricular layer of the optic tectum. Current efforts are directed at finishing the characterization of this cDNA and that of the other goldfish MLCK homologues. The molecular identification of goldfish neuronal MLCK homologues will form the foundation of future studies of actin-myosin interactions in neural development and plasticity. Supported by NIH grants EY03736 to J. Schmidt and NS30682 to B. Szaro.

699.11

THE FORWARD MOVEMENT OF GROWTH-ASSOCIATED "WAVES" ALONG THE AXONS OF CULTURED HIPPOCAMPAL NEURONS REQUIRES INTACT ACTIN FILAMENTS. G. Ruthel* and G. Banker. Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908.

We have previously described gowth-cone-like structures referred to as "waves" which periodically form at the base of axons in hippocampal cultures and travel outward along the axon to the tip, where their arrival is correlated with a spurt of growth (Ruthel & Banker, 1992, Soc. Neurosci. Abs.). Like growth cones, waves extend lamellipodia that are rich in filamentous actin. We wished to test whether maintenance of the wave structure was dependent on f-actin and, if so, whether the wave could reform after disruption. Furthermore, we were interested in where waves reformed, since reformation at the point of collapse would indicate that f-actin was required for the translocation of a wave whereas reformation at a point further along the axon would indicate that a wave could continue its forward movement independent of its f-actin structure. In order to disrupt actin filaments, we treated one-day-old cultured cells with 0.25-0.5 μg/ml cytochalasin B for 10-15 min. Waves immediately ceased their forward movement as they collapsed, forming an enlarged region on the axon or disappearing entirely. Although in some cases the halted wave did not re-emerge after removal of cytochalasin, in many cases the wave reformed and resumed its forward movement from the point where it had stopped. The reformation of waves after disruption by cytochalasin indicates that the capacity for forming a wave can persist even when the actin network that gives the wave its structure is disrupted. The lack of forward movement in the presence of cytochalasin leads us to conclude that intact actin filaments are necessary for the forward locomotion of waves along the axon.

Supported by NIH grants NS17112 and HD07323.

699.12

DIFFERENTIAL MICROTUBULE AND ACTIN ORGANIZATION IN CELLS TRANSFECTED WITH MAP2c AND MAP2d. M. Khrestchatisky*, L. Ferhat, A. Represa, A. Bernard, G. Charton, and Y. Ben-Ari. Université René Descartes, Paris V, Unité INSERM 29, 123 Bd de Port Royal, 75014 Paris France.

High and low molecular weight forms of MAP2 proteins, MAP2a-MAP2b and MAP2c-MAP2d respectively coexist with different ratios in the embryonic and postnatal rat brain. MAP2c is predominantly expressed in the immature brain. MAP2 variants are encoded by distinct mRNAs of 9 and 6 kb, both transcribed from a single gene. The MAP2d transcript comprises a 93 bp insertion encoding 31 additional amino acids in the carboxy terminal region when compared to MAP2c (Ferhat et al., Int. Neurochem. 25 pp 327-338, 1994). Developmental studies at the mRNA level show that splicing of the additional exon is regulated during ontogeny, MAP2d mRNAs being predominantly expressed over MAP2c mRNAs in all structures of the adult rat brain. We have used transient transfections in Human Embryonic Kidney cells (HEK 293) to assess the respective roles of MAP2c and MAP2d in microtubule and cytoskeletal organization, using antibodies directed against MAP2, tubulin and actin. Our results clearly demonstrate that both microtubules and actin filaments are differently organized in the presence of MAP2c and MAP2d. Microtubule bundles are thicker in the presence of MAP2c when compared to MAP2d. A similar result is obtained with actin filaments. These proteins also demonstrate different roles when cytoskeletal components are destabilized with Nocodazole or Cytochalasine D. MAP2c and MAP2d both protect microtubules from Nocodazole destabilization. In the presence of Cytochalasine D, MAP2d, but not MAP2c, protects actin filaments from destabilization.

699.13

INVESTIGATION OF MAP2C TARGETING IN DROSOPHILA NEURONS BY ECTOPIC EXPRESSION. G. Adam and A. Matus* Friedrich Miescher Institute, Basel, Switzerland CH-4002

Microtubule-associated protein 2 (MAP2) has a polarized distribution in neurons, being detectable in cell bodies and dendrites but not in axons. Tau, a related microtubule-associated protein shows the opposite pattern, being located primarily in axons. To study the molecular mechanism by which MAP2 is sorted in neurons we investigated the distribution of MAP2c, a smaller, alternatively spliced embryonic form of MAP2, in Drosophila neurons by ectopic expression. Although Drosophila neurons contain no MAP2 or tau protein mammalian MAP2 can nevertheless bind to Drosophila microtubules *in vitro* and *in vivo* (Adam et al. Neurosci. 51; 221, 1992). The GAL4-UAS system was used to express MAP2c and two truncated forms of MAP2c, CT1 and AT0. CT1 contains the caboxyl-terminal region of MAP2c including the microtubule-binding domain and AT0 contains the remaining amino-terminal segment. These polypeptides were expressed in the mushroom bodies of Drosophila brain using an expression-specific GAL4 enhancer-trap line. MAP2c was most concentrated in the cell bodies and dendrites of mushroom body neurons, while the tau protein concentration was higher in axons although also detectable in cell bodies. CT1 was distributed evenly over the extent of mushroom body neurons. These Drosophila lines will be valuable for elucidating the mechanism of MAP2c sorting in the neuronal cytoplasm.

699.14

INTRODUCTION OF ANTISENSE MAP1B BY A RETROVIRAL VECTOR DECREASES CEREBELLAR GRANULE CELL NEURITES *IN VITRO*. J. Solowska-Baird, L. Boyne , B. Kruk, B.T. Himes, D. Liu, D. Baird*, I. Fischer. Dept. of Anatomy and Neurobiology, Med. Coll. PA/ Hahnemann Univ., Philadelphia, PA 19129.

Microtubule associated protein, MAP1B, is a major structural component of growing neurites and is expressed at high levels in the parallel fibers of cerebellar granule cells early in their development. To assess the role of MAP1B in neurite elongation, a 486bp fragment of MAP1B cDNA was introduced into a retroviral vector (provided by L. Lillien) in the sense or antisense orientation. After transfection of producer cells, clones were selected which produced high titers of MAP1B sense or antisense viruses. Cerebellar granule cells were purified from neonatal rats and cultured as reaggregates in which they continue to divide. Dividing granule cells were infected with retroviruses containing either the antisense or sense MAP1B sequences. Cells were then redissociated, replated, and cultured for 48 hours. The infected cells were visualized by immunostaining for ß-galactosidase coded by the retroviral reporter gene, and MAP1B was detected via immunofluoresence. Although infection with MAP1B antisense retrovirus did not abolish MAP1B immunoreactivity, quantitative analysis of infected (ß-gal positive) granule cells showed a significant decrease in the number of cells with no neurites and in mean neurite length in cells infected with antisense MAP1B compared to those infected with sense MAP1B. Studies of *in vivo* infection of dividing cerebellar granule cells are now in progress. Supported by NS 24707, NS09486, NS33214, and NS24725.

699.15

ASSOCIATION OF MITOGEN-ACTIVATED PROTEIN (MAP) KINASE WITH MICROTUBULES. M. Morishima-Kawashima, J. Tang* and K. S. Kosik. Center Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115.

MAP kinase is a key downstream molecule in several signaling pathways. Although the *in vivo* cytoplasmic substrates of MAP kinase are unknown, the neuronal microtubule-associated proteins (MAPs) are putative substrates for this kinase. In rat primary hippocampal neurons, anti-MAP kinase antibody stained both nuclei and cytoplasm. Double staining of the detergent-extracted cells showed co-localization of MAP kinase with microtubules. Treatment of cells with microtubule disorganizing reagents, nocodazole and colchicine, retained MAP kinase staining on microtubules, suggesting that at least a part of the MAP kinase pool is associated with drug-stable microtubules. The association of MAP kinase and microtubules was also shown biochemically. MAP kinase was co-purified with microtubules by both temperature-dependent polymerization depolymerization cycles and the taxol method. Quantitative analysis indicated that 4% of MAP kinase in cytosolic extract is associated with microtubules. A fraction of MAP kinase was co-immunoprecipitated with MAP2, which is a good *in vitro* substrate for MAP kinase. Microtubules were fractionated over a phosphocellulose column and a gel filtration column to assess the co-elution of MAP kinase and MAP2. Although MAP kinase is present in both the tubulin and MAPs fractions, the isoform preference of ERK1 and ERK2 in these fractions differs. This result may reflect a functional distinction between ERK1 and ERK2.

699.16

DEVELOPMENTAL EXPRESSION AND DISTRIBUTION OF HIGH MOLECULAR WEIGHT TAU IN RAT NERVOUS SYSTEM. L. Boyne*, F. Nothias[1], A. Tessler, M. Murray, I. Fischer. Anat. & Neurobiology, Med. Coll. PA/Hahnemann Univ., Phila., PA 19129, [1]IAF-CNRS, Gif Sur Yvette, France

Tau is a microtubule associated protein that plays a role in the organization of microtubules and axonal morphogenesis. Various isoforms of tau are differentially expressed during nervous system development as the result of alternative splicing and post-translational modifications of a single tau gene. In adult PNS, tau is expressed as a high molecular weight (HMW) isoform of 110-120K. A cDNA encoding the complete 4a exon specific for HMW tau was cloned by RT-PCR into an expression vector and specific polyclonal antibodies were prepared against the fusion protein and the recombinant tau protein, respectively. The distribution of HMW tau in the developing and adult nervous system was determined by immunocytochemistry and double staining immunofluorescence with the HMW tau polyclonal antibodies and monoclonal antibodies that recognize all isoforms of tau. During development, tau was expressed in all DRG neurons, while HMW tau expression was restricted to small and medium, but not large DRG neurons and processes after embryonic day 18. After peripheral nerve lesion, distribution of tau within the DRG remained unchanged. In the spinal cord, HMW tau was present in motor neurons at postnatal day 2 and attained its adult pattern in cells bodies, axons and dendrites of ventral motor neurons by day 14. In the adult rat CNS, almost all neurons that extend processes into the PNS expressed HMW tau, including all cranial nerve motor nuclei and central processes of most sensory ganglia; of these ganglia, only the bipolar neurons of the olfactory, vestibular and spiral ganglia did not express HMW tau. Retinal ganglion cells were the only CNS neurons, whose processes remain entirely within the CNS, that expressed high levels of HMW tau. The limit and specific distribution of HMW tau in these neurons may be regulated by target-derived signals from the periphery to meet the structural requirements of these axons. Supported by VA Med. Res. Service, NIH NS24707, NS24725, NS07287, and NS09486.

699.17

EXPRESSION AND FUNCTIONAL ROLE OF TAU PROTEINS IN DIFFERENTIATED SY5Y HUMAN NEUROBLASTOMA CELLS. D. Uberti, C. Rizzini, P.F. Spano, M. Memo*, **Div. Pharmacology, Dept. Biomed Sci. Biotech., Univ Brescia, Brescia, Italy**

Although several attempts have been made, the molecular and cellular mechanisms responsible for the differential expression of various tau isoforms as well as their functional roles in morphogenesis, PHF formation, and neurodegeneration have not been completely clarified.

We have performed a series of studies on SY5Y neuroblastoma cells differentiated by retinoic acid treatment. We found that indifferentiated cells express predominantly the fetal isoform of tau, although at very low level. After retinoic acid treatment, mRNA species encoding either the fetal and the mature tau isoforms can be detected. The fetal tau isoform appeared to be much more expressed than the mature one. These cells were found resistant to glutamic acid treatment and sensitive to the neurotoxicity induced by doxorubicine (10 nM). The neurotoxic effects elicited by doxorubicine was temporally correlated with an increased of tau immunoreactivity, as measured by Alz50 antibody.

Finally, immunocytochemistry and Western analysis showed tau protein in the nucleus. The data suggest that tau is more than a microtubule associated protein, with possible role in cell differentiation-dysdifferentiation processes.

699.18

MICROTUBULE REORGANISATION IS OBLIGATORY FOR GROWTH CONE TURNING. T. Williamson, P. R. Gordon-Weeks*, M. Schachner† and J. Taylor†. D.B.R.C., King's College London, 26-29 Drury Lane, London WC2B 5RL. †Department of Neurobiology, Swiss Federal Institute of Technology, Hönggerberg, 8093 Zürich, Switzerland.

We have shown previously that growth cones of chick dorsal root ganglion cells turn at sharp substrate borders between the extracellular matrix molecules tenascin and laminin *in vitro* and grow along the laminin side of the border. To gain insight into the role of microtubules in growth cone turning, we have compared the organisation of microtubules in growth cones advancing on uniform laminin substrates with their organisation in growth cones that have encountered a laminin-tenascin border. The results suggest that a reorganisation of the microtubules occurs in growth cones turning at laminin-tenascin borders. Further evidence for the involvement of microtubule rearrangement in growth cone turning was provided by experiments in which growth cones approached tenascin borders in the presence of substoichiometric concentrations of the microtubule stabilising compound, taxol. Taxol altered the organisation of microtubules in growth cones growing on laminin by restricting their distribution to the proximal regions of the growth cone and increasing their bundling. Taxol did not stop growth cone advance on laminin. When growing in the presence of taxol, growth cones at tenascin borders were not able to turn and grow along the laminin-tenascin border, and consequently stopped at the border. Growth cones were arrested at borders for as long as taxol was present (up to six hours in these experiments) without showing any signs of drug toxicity. These effects of taxol were reversible. Together, these results suggest that microtubule reorganisation in growth cones is a necessary event in growth cone turning

699.19

TWO STRATEGIES TO PREPARE NEURAL CORTICAL CYTOSKELETON COMPONENTS FOR THE GENERATION OF MONOCLONAL ANTIBODIES AND INITIAL CHARACTERISATION OF THE IDENTIFIED PROTEINS. T. G. A. Mack, O. Schulz, S. Roth, U. Schwarz & G. E. Pollerberg* Dept. of Biochemistry, Max-Planck-Institute for Developmental Biology, D-72076 Tuebingen, Germany

Axons and growth cones possess a spectrin/actin based network closely associated with the inner side of the cell membrane, the cortical cytoskeleton. This structure serves diverse functions during axonal outgrowth, such as surface shaping, modulation of integral membrane proteins, and signal transduction. We developed two strategies to prepare material enriched for neural cortical cytoskeleton from embryonic chick nervous tissue. The first strategy combined the isolation of a membrane/cortical cytoskeleton fraction by density gradient centrifugation with an enzymatic degradation of cell surface proteins. The second strategy is based on the attachment and crosslinking of single cells to beads, allowing for the removal of the cell contents by cell disruption; only membrane/cortical cytoskeleton patches are retained on the beads.

Both strategies permitted the removal of cytoplasm, organelles and cytoplasmic cytoskeleton while retaining the cortical cytoskeleton, as shown by the detection of marker molecules. Monoclonal antibodies (mabs) generated using both preparations as immunization material were screened for recognition of submembranous structures in axons and growth cones of retinal neurons in culture. A proportion of the mabs bind to structures in the growth cone periphery, a cell region composed almost exclusively of cell membrane and cytoskeleton, and are likely to recognize components of the cortical cytoskeleton. Expression patterns and the apparent molecular weights of the detected proteins indicate that a subset of the mabs is directed against known components, whereas others appear to detect novel components of the cortical cytoskeleton. We are currently immunopurifiying and sequencing a number of proteins defined by the mabs.

FORMATION AND SPECIFICITY OF SYNAPSES: AGRIN

700.1

AGRIN MAY INTERACT WITH GROWTH FACTORS TO MEDIATE ACHR CLUSTERING AT THE NEUROMUSCULAR JUNCTION. D.F. Daggett@, D. Stone¶, K. Nikolics¶, and H.B. Peng@* @Dept. of Cell Biol. and Anat. and Curr. in Neurobiol., UNC, Chapel Hill, NC 27599, and ¶Dept. of Neurosci., Genentech, Inc., S. San Francisco, CA 94080

The clustering of acetylcholine receptors (AChRs) to high densities beneath presynaptic active zones, which is the hallmark of postsynaptic specialization at the neuromuscular junction (NMJ), appears likely to be mediated in part by the extracellular matrix protein agrin. This has been demonstrated by observations that 1) application of certain soluble agrin isoforms to cultured muscle cells induces the formation of numerous AChR clusters in the muscle membrane, 2) agrin is highly enriched at neuromuscular synapses, and 3) certain anti-agrin antibodies appear to block NMJ formation in culture. However, our previous studies have also suggested the role of endogenous, muscle-bound growth factors in forming postsynaptic specializations. Here we have coated 10 µm polystyrene beads with purified, full-length agrin isoforms (100µg/ml) to compare their ability to locally induce AChR clustering to that of growth factor-coated beads, when applied to *Xenopus* muscle cell cultures. In addition we incubated agrin-coated beads secondarily with the heparin-binding growth factor HB-GAM to look for agrin-growth factor interactions. Here we report that focally applied, agrin-coated beads cause AChR clustering at ~25% of bead muscle contacts (BMC's). However, agrin beads further incubated with HB-GAM, bind this factor as shown by immunocytochemistry and cause AChR clustering at ~80% of BMC's. Beads coated similarly with BSA and HB-GAM have no effect. These results suggest that agrin may mediate AChR clustering by interacting with muscle bound growth factors, stimulating intracellular signal transduction pathways leading to AChR redistribution. (Supported by NIH and MDA)

700.2

AXONAL TARGETING OF AGRIN IN CULTURED RAT DORSAL HORN NEURONS. G. Escher*[1], C. Bechade[2] and A. Triller[2] [1]Institute of Anatomy, Bugnon 9, 1005 Lausanne, Switzerland. [2]Ecole Normale Supérieure, INSERM CJF 9410, 75005 Paris, France.

Agrin transcripts are broadly expressed in the CNS, including in non cholinergic areas. The role of agrin in motoneurons, but not in other neurons, is well established. To approach this question, the presence and cellular distribution of agrin in non-motoneurons was analyzed in primary cultures of dorsal horn neurons, dissociated at E14 and kept in culture for 1 to 10 days; glial cells make up less than 1% of all cells.

We now show : 1) the expression of the 4 agrin isoforms B0, B8, B11 and B19 (see Ruegg et al., 1992) by RT-PCR; 2) their developmental regulation during neuronal maturation, B0 being expressed first, then - during the period of synaptogenesis, the active isoforms (B>0) are preferentially expressed; 3) that agrin-like immunoreactivity can be detected by Western blots both in the supernatant and in the cells; 4) using double immunostaining with anti-agrin antibodies (recognizing all isoforms) and anti-MAP2 or anti-Tau 1 antibodies respectively, an enrichment of agrin labeling in all axons. These data indicate that in our cultures, agrin is synthesized by almost all neurons and is targeted to axons. These observations suggest that, as in the neuromuscular junction, agrin plays a key role in pre- to postsynaptic interactions.

Supported by Swiss NSF 31-39713.93, AFM and IRME grants.

700.3

ALTERNATIVE mRNA SPLICING OF AGRIN REGULATES BINDING TO HEPARIN AND α-DYSTROGLYCAN. M. Gesemann, A. J. Denzer, V. Cavalli, A. Brancaccio[§], B. Schumacher, W. B. Adams* and M. A. Ruegg, Depts. of Pharmacology and [§]Biophysical Chemistry, Biozentrum, University of Basel, CH-4056 Basel, Switzerland

Agrin is a heparan sulfate proteoglycan with an apparent molecular weight of more than 400 kD that induces the aggregation of acetylcholine receptors (AChRs) on cultured myotubes. Several isoforms are generated as a result of alternative mRNA splicing. While the chick isoform agrin$_{A4B8}$ (4 amino acid (aa) inserted at site A; 8 aa inserted at site B, both located near the C-terminal end) induces AChR aggregation on myotubes at picomolar concentrations, agrin$_{A0B0}$ (no inserts at both sites) is inactive. A 45 kD C-terminal fragment of agrin$_{A4B8}$ (c45$_{A4B8}$) that contains inserts at both sites A and B retains high activity. A 21 kD C-terminal fragment (c21$_{B8}$) is still active, but only at 100-fold higher concentrations. We have now investigated the binding of agrin isoforms to heparin and to α-dystroglycan, a peripheral membrane protein of the dystrophin-glycoprotein complex, that is the major agrin-binding protein on muscle cells. We find that the four aa insert, Lys-Ser-Arg-Lys, at site A is required for the binding of agrin to heparin. This binding site is also functional in full-length chick agrin. Similarly, binding properties to α-dystroglycan are also affected by alternatively spliced sites. High-affinity binding to both denatured and native α-dystroglycan from chick skeletal muscle and the mouse C2 cell line was found for the inactive agrin$_{A0B0}$. The highly active isoform agrin$_{A4B8}$ binds with a 10 to 20-fold lower affinity and no binding was detected for c21$_{B8}$. These data show that binding of agrin to α-dystroglycan is not necessary for AChR aggregation and they set the stage to investigate the role of α-dystroglycan in agrin-induced AChR aggregation and to search for the signal-transducing agrin receptor.

700.4

CHARACTERIZATION OF FULL-LENGTH CHICK AGRIN, A BASAL LAMINA PROTEIN INVOLVED IN THE FORMATION OF SYNAPSES. A. J. Denzer, M. Gesemann, B. Schumacher and M. A. Ruegg*. Dept. of Pharmacology, Biozentrum, University of Basel, 4056 Basel, Switzerland.

Agrin is a protein of the extracellular matrix with a calculated molecular weight of ~ 220 kD that induces the aggregation of acetylcholine receptors (AChRs) and other molecules concentrated at the neuromuscular synapse. A 45 kD C-terminal fragment of agrin is sufficient for this activity. To investigate functional properties of the complete protein, we transfected previously described full-length chick cDNA into COS cells. However, recombinant agrin was not secreted from the cells. Here we describe a 5' extended construct, probably encoding complete chick agrin, that is secreted from COS cells. The novel sequences were found by primer extension studies and isolation of the agrin gene. They extend the coding sequence at the 5' end by ~ 350 bp. Protein sequence encoded by this extension is highly homologous to a 15 amino acid peptide of a heparan sulfate proteoglycan (HSPG) isolated from bovine kidney (Hagen et al. (1993) JBC 268, 7261). Recombinant agrin is active in inducing AChR aggregation depending on the splice variant. It has an apparent molecular weight between 400 and 600 kD and carries glycosaminoglycan side chains attached to the N-terminal part that are characteristic for a HSPG. Polyclonal antisera detect agrin-like protein in tissue homogenates that also has an apparent molecular weight between 400 and 600 kD. In addition, we have found a novel site of 7 amino acids at the N-terminal part that is alternatively spliced. While motor neurons of 5 day old chick embryos synthesize agrin mRNA encoding the 7 amino acid insert, muscle cells predominantly express transcripts that lack this insert. These experiments will allow us to investigate binding properties of recombinant agrin to possibly get insights into other functions of this molecule.

700.5

THE ROLE OF DIFFERENT AGRIN ISOFORMS IN SODIUM CHANNEL CLUSTERING. A. A. Sharp[1]*, J. T. Campanelli[2], R. H. Scheller[2] and J. H. Caldwell[1]. [1]Dept. of Cellular and Structural Biology, Univ. of Colorado Health Sciences Ctr., Denver, CO, 80262; [2]Beckman Ctr., Stanford Univ. Med. Ctr., Stanford, CA, 94305.

Sodium channels and ACh receptors are expressed at particularly high concentrations at the neuromuscular junction. Agrin is known to play a key role in the aggregation of ACh receptors. Five isoforms of agrin are known to be differentially expressed at different developmental stages as well as by different tissues. The isoforms of agrin also differ in their ability to aggregate ACh receptors. Lupa and Caldwell (J. Cell Biol., 1991, 115:765) have shown that *Torpedo* agrin presented in the media of primary rat muscle fiber cultures was unable to cluster sodium channels. The inability of agrin to cluster sodium channels in that study may have been the result of presenting the wrong isoform of agrin to the cultures. In this study we are testing the ability of different isoforms of agrin to aggregate sodium channels. Adult rat muscle fibers from the flexor digitorium brevis are dissociated and co-cultured with CHO cells that have been transfected with various isoforms of agrin and express it on the extracellular side of the membrane (Ferns et al., 1993, Neuron, 8:1079). Sodium channels are detected with immunocytochemical techniques using a polyclonal antibody to sodium channels. CHO cells expressing the A4B8 isoform of agrin cause clustering of sodium channels when attached to the perijunctional and near-perijunctional regions of the fibers after one day in culture. CHO cells attached more distally do not cause aggregation at a level detectible by this process. Wild type CHO cells do not cause aggregation of sodium channels. We are also testing the ability of CHO cells expressing the A4B19, A4B11 and A4B0 to aggregate sodium channels.

700.6

AGRIN FROM NG108-15 INDUCES ACETYLCHOLINE RECEPTOR AGGREGATION ON PRIMARY RAT *MYOTUBE CULTURES*. S. Pun, K. L. So and K. W. K. Tsim*. Dept. of Biology, The Hong Kong University of Science and Technology, Clear Water Bay Road, Kowloon, Hong Kong.

Agrin, a protein isolated from the synaptic basal lamina, directs the formation of the postsynaptic apparatus on developing and regenerating neuromuscular junctions. The full-length cDNAs for agrin have been cloned in chick and rat species; both are ~8 kb in size and encoded proteins have a deduced molecular weight of over 200 kDa. The expression of agrin is not restricted to the motor neurons of spinal cord; it is also expressed in other tissues including the brain. We study the expression of agrin and its isoforms in a neuroblastoma x glioma cell line (NG108-15). These cells have the ability to induce the aggregation of acetylcholine receptors (AChRs) on the cultured myotubes. The co-culture of NG108-15 cells and primary rat myotubes resulted in ~10-fold increase in the AChR aggregates on the surface of myotubes. Using an agrin-specific antibody, the agrin protein of ~200 kDa was identified in NG108-15 cells by immunoblotting. Its corresponding transcript of ~8 kb in size was detected by Northern blot analysis using agrin cDNA as a probe. Expression of no amino insert at z site of agrin mRNA in NG108-15 cells was also demonstrated using polymerase chain reaction. To demonstrate the aggregation of AChRs is due to the secreted agrin from NG108-15 cells, antisense construct of agrin cDNA was transfected into the NG108-15 cells. The NG108-15 cells induced-AChR aggregation on cultured rat myotubes was blocked by such antisense cDNA transfection. These studies provide a direct evidence that the NG108-15 cell-induced AChR aggregation on cultured primary rat myotubes is mediated by agrin.

700.7

DOMAINS OF AGRIN THAT INDUCE ACHR PHOSPHORYLATION. T. Meier, M. Gesemann, V. Cavalli, M.A.Ruegg and B.G.Wallace*. Dept. of Physiology, Univ. Col. Hlth. Sci. Ctr., Denver, CO 80262 and Dept. of Pharmacology, Biozentrum, Univ. of Basel, CH-4056 Basel, Switzerland.

Differentiation of the postsynaptic apparatus at developing and regenerating vertebrate skeletal neuromuscular junctions is triggered by agrin. Agrin is a 400-600 kD heparan sulfate proteoglycan with several regions homologous to domains found in other extracellular matrix proteins. In addition, several isoforms of agrin have been identified that arise by alternative splicing of a single agrin gene. When added to myotubes in culture, agrin induces the formation of specializations at which acetylcholine receptors (AChRs) and other components of the postsynaptic apparatus accumulate, and also induces tyrosine phosphorylation of AChRs. Treatments that block agrin-induced protein tyrosine phosphorylation prevent AChR aggregation, suggesting that phosphorylation may play a role in receptor aggregation. Recent reports indicate that agrin binds to α-dystroglycan and it has been proposed that this binding mediates AChR aggregation. To test further the relationship between tyrosine phosphorylation and receptor aggregation and to determine if interaction of agrin with α-dystroglycan is required for AChR phosphorylation, we have compared the ability of fragments of various agrin isoforms to trigger AChR phosphorylation and/or aggregation. Results indicate that the same domains and isoforms of agrin that induce AChR aggregation also cause AChR tyrosine phosphorylation, while domains that mediate binding of agrin to α-dystroglycan are not required for either AChR aggregation or phosphorylation.

700.8

NGF DEPENDENT REGULATION OF AGRIN GENE EXPRESSION IN PC12 CELLS. M. A. Smith[1], L.T. O'Connor*[1], G. Fanger[2], and R.A.Maue[2]. [1]Dept. Anat. and Neurobiol., University of California, Irvine, CA 92717, and [2]Dept. Biochem. and Physiol., Dartmouth Med. Schl., Hanover, NH 03755.

Agrin is an extracellular matrix protein believed to play a role in the formation and maintenance of chemical synapses. Expression of alternatively spliced agrin mRNAs that encode proteins that differ in their acetylcholine receptor aggregating activity is both cell specific and developmentally regulated. For example, in neurons, agrin11 transcripts are elevated during early development whereas agrin 8 mRNA is typical of more mature cells. To better understand the cellular mechanisms that control agrin gene expression we have analyzed changes in the level of agrin mRNA and pattern of alternative splicing in PC12 cells in response to growth factors. When compared to control cells, treatment with NGF for 7d caused a significant (2.4 fold) increase in total agrin mRNA expression. RT-PCR analysis shows that in the absence of NGF, PC12 cells express predominantly agrin0 mRNA that encodes protein with low acetylcholine receptor aggregating activity, whereas transcripts encoding agrin isoforms with high activity (agrin8, -11, -19) are at or below detection. NGF treatment results in a marked increase (>25 fold by 7 days) in agrin8 mRNA expression. The selective increase in agrin8 mRNA is consistent with the neuronal differentiation of PC12 cells that occurs in response to NGF. In contrast, preliminary results indicate that the pattern of agrin alternative splicing is not affected by treatment with either insulin or EGF. Analysis of *Ras* deficient PC12 sublines (courtesy of G. Cooper, Dana Farbe Cancer Inst., Boston, MA) suggests further that the NGF induced increase in agrin8 mRNA is a *Ras* dependent response. The results imply that in addition to changes in gene transcription and mRNA stability, NGF can influence alternative splicing and raise the possibility that NGF and perhaps other growth factors may influence agrin gene expression *in vivo*. Supported by NS33213 to MAS and NS28767 to RAM.

700.9

REGULATION OF AGRIN GENE EXPRESSION DURING DEVELOPMENT OF MOUSE THALAMUS AND SOMATOSENSORY CORTEX. Z. Li[1], J.L. Massengill[1], D.K.O'Dowd[1,2] and M.A. Smith*[1]. Depts. of [1]Anat. and Neurobiol. and [2]Dev. and Cell Biol., University of California at Irvine, Irvine, CA 92717.

Agrin mRNA is expressed by many neuronal populations in developing and adult rat brain, suggesting a role for agrin in the formation and maintenance of synapses in the mammalian CNS. To investigate this possibility we have begun a detailed study of agrin gene expression in the mouse thalamocortical system during the first 3 weeks of postnatal development, a period when functional connections between thalamus and cortex as well as within cortex form and mature. Whereas all four agrin mRNAs resulting from alternative splicing at the z region are present in RNA from ventrobasal thalamus, their relative abundance changed during development. Agrin0 mRNA increased steadily from approximately 40% of total agrin mRNA at birth (P0) to 80% at P20. Agrin8 transcripts were first detected at P5, with maximal levels (≈10%) occurring around P16. Agrin11 mRNA was present only during P0-P4 with a peak (≈4%) at P1. Agrin19 transcripts represented about 50% of total agrin mRNA at P0, declined to approximately 40% by P3 followed by a further decrease to about 10% between P7 and P20. A similar pattern of agrin mRNA expression was also observed in somatosensory cortex. In parallel studies *in vitro*, we observed that functional connections formed between dissociated P0 cortical neurons during the first two weeks in culture and that temporal changes in agrin8, -11, and -19 mRNAs were similar to those between P0-P14 *in vivo*. Thus, developmental changes in agrin gene expression *in vivo* and *in vitro* are correlated with formation of functional synaptic connections, consistent with a role for agrin in synaptogenesis in the mammalian CNS. Supported by NS07351 to ZL, NS33213 to MAS and NS30109 to DO'D.

700.10

Agrin is Adhesive for Motor Neurons and Inhibits Neurite Outgrowth. J.A. Campagna* and J.L. Bixby. Dept. of Molecular and Cellular Pharmacology, Univ. Of Miami Sch. of Med., Miami, FL 33101.

A number of proteins are concentrated in the synaptic basal lamina at the neuromuscular junction (NMJ). Evidence suggests that some of these proteins are important for motor neuronal axon growth and subsequent synaptogenesis. s-laminin (s-LN) is a 200 kD protein that is highly enriched in synaptic basal lamina, is selectively adhesive for motor neurons, and can act as a "stop signal" for growing axons; the tripeptide motif LRE (leu-arg-glu) is implicated in these functions. (Hunter & Sanes, J. Neurosci 11,3960, 1991;Porter *et al.*, Neuron 14, 549, 1995) A second protein enriched in synaptic basal lamina and containing an LRE motif is agrin, which has previously been shown to induce several aspects of postsynaptic differentiation at the NMJ. To test the hypothesis that the LRE motif in agrin is also a motor neuron "stop signal", we have grown chick ciliary ganglion (CG) neurons on substrates of agrin-expressing CHO cells. CG neuron growth cones extending on a fibronectin substrate stop growing upon contact with $agrin_{19}$-expressing CHO cells, but not untransfected CHO cells. In a short-term adhesion assay, CG neurons adhered four times as well to agrin-expressing cells as to untransfected CHO cells. Our results are consistent with a general role for LRE-containing basal lamina proteins in directing motor neurons to muscle, and suggest that agrin's role at the NMJ is not limited to actions on the myotube.

700.11

IDENTIFICATION OF A NOVEL AGRIN ISOFORM PREFERENTIALLY EXPRESSED IN NON-NEURONAL CELLS. G. Tsen[1], A. Napier[1], W. Halfter[2], and G. J. Cole[1]*. [1]Neurobiotechnology Center and Dept of Cell Biology, Neurobiology and Anatomy, The Ohio State Univ., Columbus, OH 43210. [2]Dept of Neurobiology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

A novel isoform of agrin was identified based on the isolation of an agrin cDNA from E9 chick brain that lacks 21 bp in the N-terminal encoding region of the agrin mRNA. RT-PCR of E9 chick brain mRNA confirmed the existence of this agrin isoform in brain, but the novel splice variant represents only a minor fraction of agrin mRNA in brain. However, upon the analysis of chick astrocyte mRNA, smooth muscle mRNA, and cardiac muscle mRNA by RT-PCR, this novel isoform was shown to be the predominant agrin isoform in these non-neuronal cell populations. We extended our analyses to examine the expression of this agrin mRNA isoform during chick development, and showed that in brain this novel agrin isoform is up-regulated with brain development, consistent with the increase in glial number during brain development, while the agrin isoform that does not undergo splicing and thus contains the 21 bp exon is down-regulated. We also showed that in heart both agrin isoforms are down-regulated during development. Because the 21 bp exon is inserted in the region of chick agrin which encodes the putative signal sequence of agrin, with the signal peptidase cleavage site immediately preceding the putative first amino acid of the mature protein being deleted as a result of splicing, the presence or absence of this alternatively spliced exon may regulate differential processing of the agrin protein in neuronal and non-neuronal cells, repectively.
Supported by NIH grant NS33981.

FORMATION AND SPECIFICITY OF SYNAPSES II

701.1

THE DEVELOPMENT OF SYNAPSES IN BARRELS OF MOUSE PRIMARY SOMATOSENSORY CORTEX. E.L. White*, D. Lev and E. Weinfeld Center for Brain Research, Faculty of Health Sci., Ben-Gurion Univ., Beer Sheva, ISRAEL.

As part of a comprehensive study of the development of synaptic patterns in mouse PMBSF barrels, the distribution of synapses was assessed in EM montages taken of thin sections cut tangentially through the mid-layer IV level of barrel D4, usually, but also of adjacent barrels D5 or C4 at (to date) P6, P9, P11, P13 and at 65 days of age (P1=the first 24 hours after birth). Barrel hollows, sides and septa were identified in 40μm thick sections cut from unembedded, aldehyde perfused, osmicated brains. From P6, dark barrel hollows are clearly recognizable with the light microscope due to the high osmophilia of the processes (primarily axons) within them; by P11 the hollows are somewhat more faint due to their increasing content of less osmophilic dendritic branches. Most somata received symmetrical synapses only. At P6, 111 profiles of 113 somata received no synapses, the remaining profiles received one each. At P9, 67% of 135 profiles formed 1 (usual) or more synapses; at P11, 46% of 167 profiles formed 1 or more; at P13, 90% of 77 formed 1 or more (53% 2 or more) and in adult, 89% of 143 profiles formed 1 or more synapses (63% 2 or more). Synapse counts at all ages, whether on somata or in the neuropil, were similar for equivalent areas of wall vs. hollow. Exclusive of somatic synapses, P6 neuropil (hollow samples>14,000μm^2/age; in wall, primarily septa>4,000μm^2/age) contained 1.5 synapses/100μm^2, P9 had 4.3 and the adult 9.7. Supported by Israel Acad. of Sciences 618/93 to E.L.W.

701.2

TOPOGRAPHY OF THE ECTOPIC RETINAL PROJECTION TO OLFACTORY CORTEX (OC) IN ADULT *RANA PIPIENS*. Scalia, F.*, W. Su, E. Harris, S.M. Galoyan, S. Eisner, and J.Y. Lettvin, Dept. of Anat. & Cell Biol., SUNY HSCB, Brooklyn, NY 11203 and Media Lab., MIT, Cambridge, MA 02139.

The prominent electrical responses visually elicited in the superficial neuropil (SN) of OC 6 mo after translocation of a regenerating optic nerve into the telencephalon (T) were evocable only from the frontal visual field, but a spatial map of the responses was not observed (Neurosci. Abs., Scalia et al., '94). Transients of this type and size are recorded in the SN of the optic tectum with presentation of prey-mimetic stimuli in any sector of the visual field (Grant and Lettvin, Brain Res., '91), but are never found in normal OC. Anatomical data on the sectorial origin of the ectopic fibers was sought to investigate the basis of the frontal-field-dominance in this projection. Mixed injections of biotin-dextran (BDA) and ^{3}H-amino acids were given intravitreally immediately after cutting across a defined sector of the retinal nerve fiber layer in frogs surviving 6-8 mos after transplantation of the optic nerve into T. The injected specimens survived an additional 3 days to allow time for anterograde migration of the BDA in the cut retinal axons and transport of the ^{3}H-label by the uninjured ganglion cells in the complementary areas of the retina. BDA-labeling was examined both by LM and EM. When the cuts were made across temporal sectors of the retina, which image the frontal-field, the BDA-labeled terminal plexus in the experimental animals occupied the posterodorsal half of the ectopic projection-field. After nasal retinal lesions, the BDA-labeled terminals were distributed in the more anteroventral part of the projection-field. The pattern of radiolabel in autoradiographs of adjacent sections was complementary to the BDA-labeling, but with significant overlap in the fields defined by the two tracers. Thus, the ectopic projection arises from both the temporal and nasal halves of the retina, but only the former becomes capable of evoking the large-amplitude responses. (Supported by NIH Grant EY05284)

701.3

ASYMMETRIC NASAL/TEMPORAL EXPRESSION OF AN ALTERNATIVELY SPLICED GENE WITHIN THE DEVELOPING RETINA. W.M. Jurney, D.R. Foltz, D.J. Selski and S.C. McLoon*. Univ. of Minnesota, Minneapolis, MN 55455.

Development of the topographic pattern of axonal connections from the eye to the brain is believed to involve molecules expressed asymmetrically across the developing retina that mark cell position. In an attempt to identify such molecules, differential kinetic enrichment was employed to isolate asymmetrically expressed mRNAs in the nasal-temporal axis of the embryonic chick retina. A PCR amplified cDNA representing a mRNA enriched in the nasal side of the retina was used to screen an embryonic retinal cDNA library. Seven clones were isolated. Restriction analysis suggested that these clones share a 2kb "conserved" region, and each had a "variable" region of up to 2kb. A probe prepared from a fragment of the conserved region of one clone hybridized to all seven clones. Two separate fragments from the variable region of one clone hybridized to subsets of the seven clones. RNA blot analysis suggested that the conserved region is expressed equally on the two sides of the retina, while transcripts containing either of two fragments from the variable region were more abundant on the nasal side of embryonic retina. These results suggest the existence of an alternatively spliced gene, the products of which are asymmetrically expressed across the developing retina. Comparison of a partial sequence from one clone suggested that it is a previously unreported gene. (Supported by NIH grants EY05371 and EY07133.)

701.4

QUANTITATIVE DISTRIBUTION OF GABA-IMMUNOREACTIVE TERMINALS AFTER SENSORY DEPRIVATION IN THE RAT BARREL CORTEX. C. Beaulieu* and C.Crevier. Dept Pathology and CRSN, Université de Montréal, Montréal, (Qc) Canada.

We have previously shown that neonatal sensory deprivation leads to a 50% reduction in the numerical density (N_V) of synaptic contacts formed by GABA(+) boutons in layer IV of deprived barrels as compared to controls. To estimate whether this change was due to an actual loss of GABA(+) axon terminals, we calculated their number in layer IV of the barrel cortex of rats which had their vibrissae on the right face continuously removed from birth.

In long series of ultrathin sections from layer IV, we identified and followed all GABA(+) boutons through their entire length. By applying the disector method, we calculated that the N_V of GABA(+) boutons was 25% lower ($p<0.01$) in sensory-deprived (48±7 million per mm^3) than in control (64±10 million/mm^3) barrels. Thus, the decrease of contact number in sensory-deprived cortex is due partially to a loss of GABA terminals. By dividing the N_V of synaptic contacts (determined in the previous study) by that of boutons, we determined a contact-to-bouton ratio. On average, a GABA(+) bouton of the deprived barrels formed 1.9 contacts while this ratio reached to 2.5 in the controls. This suggests that the decrease of contacts could be due not only to a loss of GABA(+) terminals but also to a decrease of the number of synaptic contacts formed by each bouton. However, it is also possible that the decrease of synaptic contacts was consequent to a preferential loss of a subpopulation of GABA(+) terminals making on average, a high number of contacts. This is currently under investigation. (Supported by MRC, FRSQ, and FCAR)

701.5

GABA IMMUNOCYTOCHEMICAL ANALYSIS OF CEREBELLAR EXPLANT CULTURES FROM THE LURCHER MUTANT AND WILD-TYPE MOUSE. Keith W.T. Caddy*[1], and Martin L. Doughty[2]. [1]Dept. of Physiology, University College London, WC1E 6BT; [2]Lab. de Neurobiologie du Développement, Univ. P. & M. Curie, 75005 Paris.

Lurcher is an autosomal dominant murine mutation. Lurcher heterozygotes (+/Lc) lose all their cerebellar Purkinje cells by adulthood (Caddy and Biscoe, 1979. Phil. Trans R. Soc. B. *287*: 167-201). Chimeric analysis has shown that the primary target of the +/Lc mutation is the cerebellar Purkinje cell (Wetts and Herrup, 1982. J. Embrol. Exp. Morph. *68*: 87-98). Purkinje cells from the +/Lc mutant mouse survive and differentiate in long-term cerebellar explant cultures, indicating that +/Lc Purkinje cell death is non-autonomous (Doughty et al., 1995. J. Comp. Neurol. *in press*). The neuronal environment and synaptic investment of Purkinje cells in +/Lc and +/+ (control) cerebellar explant cultures were examined using γ-aminobutyric acid (GABA) immunocytochemistry. Cerebellar explants from 2 days postnatal (P2) +/+ and +/Lc mutant mice were grown for 15 days *in vitro* before being processed for GABA immunocytochemistry. GABA-immunolabelled explants were examined in the light and electron microscope. GABA-immunostained Golgi and basket and/or stellate cells were scattered throughout the +/+ and +/Lc cerebellar explant cultures and there was no *in vivo*-like arrangement of the immunoreactive somata. Qualitative observations did not indicate any difference in the numbers and distribution of the GABA-immunostained cells in the +/+ and +/Lc cultures. Electron microscopical analysis revealed a similar, complex distribution of the GABA-immunostain in the +/+ and +/Lc cerebellar explant cultures. The somata of Golgi and basket and/or stellate cells were GABA-immunostained, whereas their neurites had a varied immunoreactivity: GABA-immunostained and GABA-immunonegative axons formed synapses directly with the shafts of spiny Purkinje cell dendrites; whilst all the synapses on the dendritic spines of Purkinje cells were from GABA-immunonegative parallel fibres. (Supported by The Wellcome Trust).

701.6

DESCRIBING AND GENERATING COMPLEX DENDRITIC MORPHPOLOGIES USING L-SYSTEMS

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We describe the use of l-systems for both the generation and description of complex dendritic mophologies. L-systems provide powerful language in which both the topology and geometry of complicated branching structures can be described in detail using few parameters. Such a language permits the exploration several important questions using computational models. Two of these are: "How does dendritic morphology affect the computations performed by single neurons?" and "What rules govern the pruning of dedritic arborizations during development?". This formalism also provides a useful descriptive tool for experimentalists. An inference algorithm takes data on the mophology of actual neurons and outputs an l-system which generates that mophology to within a specified error. The l-system which generates the morphology can be used as a much more complete and compact quantitative description of morphology than other measures that have been used to date for this purpose.

701.7

STABILISING NEUROMUSCULAR CONTACTS INCREASES MOTONEURONE SURVIVAL AFTER NEONATAL NERVE INJURY IN RATS. D. Harding, L. Greensmith, A.L. Connold, G. Vrbova, and S. Tuel*, Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, U.K.

After a sciatic nerve crush at birth the rat soleus muscle is permanently weak. The weakness is caused a) by loss of a proportion of its motoneurones and b) by the failure of the surviving motoneurones that are successful in reaching the muscle and reinnervate it 7 days later to expand their peripheral field. Here we attempted to maintain new neuromuscular contacts by applying leupeptin, an inhibitor of the calcium activated neutral protease. This protease appears to be involved in the loss of neuromuscular contacts during development and sprouting. This study shows that in muscles treated with leupeptin the reduction in weight and force after nerve crush at birth was significantly less than in those that were untreated. Moreover, the leupeptin treated muscles had significantly higher numbers of motor units than untreated muscles. In the leupeptin treated animals the number of remaining motor units in the soleus muscle was 7.6 ± 0.7 (S.E.M., n=8), whereas in the NaCl treated animals soleus had only 3.4 ± 0.4 (S.E.M., n=10) motor units. Thus, protecting regenerating neuromuscular junctions during early stages of reinnervation rescues motoneurones and improves muscle recovery.

701.8

REGULATION OF N-TYPE CALCIUM CHANNEL DISTRIBUTION IN ISOLATED HIPPOCAMPAL NEURONS. R.K. Lartius[1,*], L.-E. Trudeau[1], R. Doyle[1], D.R. Witcher[2], K.P. Campbell[2] and P.G. Haydon[1]. [1]Dept. of Zoology and Genetics, Iowa State University, Ames, IA 50011; [2]Howard Hughes Medical Institute, Dept. of Physiology and Biophysics, Univ. of Iowa, College of Medicine, Iowa City, IA 52242.

The influx of calcium through voltage dependent N-type calcium channels stimulates vesicle fusion during synaptic transmission. Little is known, however, regarding the cues that regulate the expression of these channels and their localization during development. For example, is neuron-neuron contact required for normal N-type calcium channel expression, distribution and function? To address this question, hippocampal neurons were grown under conditions which resulted in either isolated neurons or neurons with neuron-neuron contacts. Differences in the expression of the N-type calcium channel subunit α_{1B} were then compared using immunocytochemistry. At 8 days in culture (DIC) neurons that were allowed neuron-neuron contact displayed diffuse α_{1B} immunoreactivity in the soma as well as immunoreactivity concentrated in punctate structures within neurites. Isolated neurons also expressed α_{1B} at 8 DIC, but in contrast, the distribution pattern was diffuse and non-punctate in neurites. This distribution pattern was comparable to an "immature" pattern observed for 4 day neurons which were allowed neuron-neuron contacts. To test whether the N-type calcium channel subunits observed by immunocytochemistry form functional calcium channels in neurons displaying the "immature" pattern, whole-cell patch clamp experiments were performed. By 4 DIC a clearly detectable ω-CgTx-sensitive calcium current was present (24.6±3.5% block, n=6). These data suggest that N-type calcium channel subunits are assembled into functional channels to be later redistributed at sites of synaptic contact in response to signals provided by target neurons.

701.9

SYNAPSE-FORMATION IN A GLIA-FREE CULTURE OF DISSOCIATED CHICK CEREBRAL NEURONS. T.Taguchi*, X.-X. Bo+, H.Taniguchi, K. Kiyosue+, S. Kudoh+, S. Murase. Dept. Organic Materials, Osaka Nat'l. Res. Inst., Ikeda 563, Japan, +Fac. Eng. Sci., Osaka Univ. Toyonaka 560, Japan.

The roles of glia in brain have been regarded as important for not only structural and nourishing support of neurons but also active modulation of neuronal functions. In some recent reports, it was shown that glial cells have a potential to mediate messages from a postsynaptic cell to presynaptic one. To elucidate precise roles of glia in neuronal systems, a glia-free neuronal culture is a useful system. Although inhibitors of cell proliferations such as cytosinearabinoside has so far been utilized, they were found to have toxic effects on neuronal growth and survival. Therefore, we have developed a novel procedure to prepare a glia-free neuronal culture system by adding certain peptides containing a cell adhesion-relating sequence such as RGD into culture medium. The synapse-formation in the culture was found to proceed in a similar way to that in the glia-containing one. To form synaptic connection, a growth cone of an axon is thought to recognize a specific target molecule such as cell adhesion molecules. The results obtained in the culture indicate that a protein that recognizes RGD sequence is not involved in the synaptogenesis in the culture.

701.10

EXPOSURE TO PHENCYCLIDINE ALTERS MOTILITY AND THE DEVELOPMENT OF IDENTIFIED NEURONS IN THE CHICK EMBRYO SPINAL CORD. B. Mendelson* K.M. Martin and M.K. Bonner. Dept. of Anatomy, University of Arkansas for Med. Sci., Little Rock, AR 72205.

Exposure to phencyclidine (PCP) during development often produces detrimental effects including long lasting alterations in motor function. PCP is known to be a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor. We have previously shown that embryonic exposure to another NMDA-receptor antagonist, MK-801, disrupts the development of cutaneous nerve projections in the chick spinal cord (Dev. Brain Res., 82: 152-166). To determine if exposure to PCP also induces alterations in spinal cord development, chick embryos were exposed to PCP during the period when primary sensory afferents form connections in the spinal cord. Groups of chick embryos were treated daily with either: 1.0 or 10.0 mg/kg of PCP in sterile Tyrode's solution (physiological saline, ST). Control embryos were treated with equivalent volumes of ST. Applications of PCP or ST began in embryonic day 5 (E5) and continued until the animals were sacrificed at E14. Embryonic motility was assayed daily at 5 time points: 15 min before drug exposure, and, 15 min, 1 hr, 4 hr and 7 hr after drug exposure. Both doses of PCP induced significant decreases in motility 15 min following PCP administration. Motility recovered to control levels by 23.75 hr after drug delivery. Following sacrifice of the animals at E14, the pattern of cutaneous nerve projections in the dorsal horn was determined by applying fluorescent tracers to identified cutaneous nerves. PCP-treatment induced a dramatic alteration in the organization of cutaneous nerve projections. The central projection formed by the lateral femoral cutaneous nerve, which normally occupies only the ventral portion of lamina 2 in lumbosacral segment 1, spread to occupy almost the entire cross sectional area of lamina 2. These data suggest that some of the detrimental effects of embryonic PCP exposure are due to inappropriate synapse formation by primary sensory afferents in the spinal cord dorsal horn. (Supported by AA09205).

701.11

FUNCTIONAL CONNECTIVITY IN THE COCULTURED RAT TRIGEMINAL SYSTEM. E. Günhan-Agar*, W. Guido, F.-S. Lo and R.S. Erzurumlu. Dept. Anatomy and Neuroscience Center of Excellence, LSU Med. Ctr. New Orleans, LA, 70112.

In explant cocultures of embryonic rat trigeminal ganglion (TG) with same age whisker pad and more mature brainstem explants (slices through the brainstem trigeminal nuclei ,BSTC), TG axons grow and arborize in both targets (Erzurumlu et al., PNAS, 1993). Here we further characterize the morphological and cellular aspects of this connectivity. DiI-labeled trigeminal axons form small, well-defined arbors within the BSTC. Biocytin-filled BSTC neurons have small, irregular shaped soma, smooth dendrites, and simple dendritic branching patterns. Of interest was to examine the membrane properties of BSTC neurons and determine whether TG input gives rise to postsynaptic responses. Intracellular recordings indicate that BSTC neurons exhibit overshooting action potentials, and at least two after hyperpolarizations, an early fast one (Ic) and a late slow one (Iahp). These dampen the rate of depolarization and promote graded responses with increasing stimulus strength, a feature that may help BTSC neurons sort out various presynaptic inputs during development. Many BTSC cells have low threshold (LT) Ca^{++} spikes. Unlike those seen in mature neurons, these are relatively small and lack burst firing. The presence of LT spikes at this age provides cells with large amounts of Ca^{++} during active dendritic growth and synapse formation. Stimulation of TG explants leads to postsynaptic responses in BSTC neurons. Some cells have complicated responses: at least two EPSP components (an early fast and slow late one) and one relatively fast IPSP. In some cases postsynaptic responses trigger LT spikes. Our results show that functional synaptic connectivity is established in trigeminal cocultures, providing a unique means to study the development of a highly ordered sensory pathway on a cellular level. Supported by NSF 9396270 (W.G) and N.I.H. NS32195 (R.S.E.)

701.12

CHARACTERISTICS AND FUNCTIONS OF CAJAL-RETZIUS CELLS IN ORGANOTYPIC SLICE CULTURES OF HIPPOCAMPUS AND NEOCORTEX. J.A. Del Río [1], E. Soriano [1] H. Supèr [2], M. Frotscher [2], B. Heimrich [2,]* [1] Dept. of Cell Biology, Faculty of Biology, University of Barcelona, Diagonal 645, 08028 Barcelona, Spain. (2) Institute of Anatomy, University of Freiburg, P.O.B. 111, D-79001 Freiburg, Germany.

Cajal-Retzius cells are the most typical representatives of the marginal zone-layer I in the neocortex and hippocampus. These transient neurons disappear during development, and their functions remain to be elucidated. Taking advantage of calretinin-immunocytochemistry, a marker for Cajal-Retzius cells in the murine cortex (Del Río et al., Cerebral Cortex, 5:13), we investigate here the features of these cells in slice cultures "in vitro", and address their developmental roles. Whereas Cajal-Retzius cells in cultures taken from P0-P2 neocortex undergo cell degeneration with a temporal sequence similar to that "in vivo", cells in the hippocampus persist after long incubation periods. Since we have proposed that Cajal-Retzius cells in the hippocampus may be involved in the attraction and targeting of developing entorhinal afferents (Supèr and Soriano, J. Comp. Neurol., 344:101), we have analyzed the development of the entorhino-hippocampal connections in co-cultures of entorhinal cortex and hippocampus. Anterograde tracing of entorhinal afferents with biocytin show that ingrowing axons overlap and are intermingled with Cajal-Retzius cells, establishing synaptic contacts. Further, cells in these co-cultures undergo cell degeneration shortly after the ingrowth of entorhinal afferents. These results suggest that Cajal-Retzius cells might act as guides for developing entorhinal axons, which might in turn influence the degeneration of these transient cells. Efforts are currently underway to determine whether the ablation of Cajal-Retzius cells in organotypic co-cultures alters the pattern of termination of entorhinal afferents.

701.13

RECORDING ELECTRICAL ACTIVITY FROM HELISOMA BUCCAL GANGLIA AND CENTRAL RING GANGLIA CULTURED ON A PLANAR MULTIELECTRODE ARRAY. I. Kim*[1], Y. Jimbo[2], and A. Kawana[2]. [1]Dep. of Biology, University of Southwestern Louisiana, Lafayette, LA 70504, and [2]NTT Basic Research Lab., Atsugi-shi, Kanagawa,243-01 Japan.

Recent advances in techniques for multisite recording and stimulation from multiple extracellular microelectrodes allow simultaneous and chronic observation of electrical activity of substantial populations of neurons. By using the planar electrode array (PEA) system, the long term profile of electrical activity has been recorded from both dissociated and organotypically cultured rat cortical neural networks, and the periodical electrical stimulation through electrodes induced bursting response in silent cultures of rat cortical neurons (A. Kawana et al., Soc. Neurosci. 1994).

The merits of simultaneous chronical multi-site recording and stimulation are utilized to study the dynamics of functional connectivity in cultured Helisoma neural networks. In snail Helisoma, several observations suggested that patterned neuronal activity in the buccal ganglia (BG) for feeding behavior receives descending excitatory and inhibitory afferents from central ring ganglia (CRG: 5 pairs of ganglion). In order to study the dynamics of functional connectivity and electrical coupling between buccal pattern generator in BG and each ganglion of CRG, the neural network among BG and each ganglion of CRG was generated on a planar lithographically-patterned arrays of 64 electrodes. The electrodes (each size: 50x50 μm) were coated with platiunm black, and separated by 250 μm distance (Y. Jimbo et al, IEEE Trans. BME). Isolated BG and CRG were trypsinized and ganglionic conective tissues were desheated with an electrically sharpened microknife. The desheated masses of BG and CRG were placed and cultured for the formation of neural networks on the PEA. Each mass of ganglion covered around 4-16 electrodes, and the detected amplitude of electrical activity were ranged around 40 μV. The dynamical changes of electrical activity of the desheathed mass of ganglion were simultaneously recorded from multiple electrodes, and analyzed.

701.14

PERFORATED PATCH RECORDING OF ACTION POTENTIALS IN PITUITARY MELANOTROPES OF NEWBORN RATS. J.C. Gomora*, G. Avila, A. Navarrete, A. Marin and G. Cota. Dept. of Physiol., Biophysics and Neurosciences, Cinvestav, Mexico, D.F. 07000.

We have previously reported that neonatal rat melanotropes generate a pattern of voltage-dependent ionic currents characteristic of excitable cells. We have also shown that sodium and calcium currents in these cells undergo a marked inhibition concomitant with the onset of dopaminergic innervation (*Soc Neurosci Abstr* 20:1685, 1994). In the present study, we have used the nystatin perforated patch recording technique to investigate whether non-innervated neonatal melanotropes are electrically excitable. Cells were obtained from 2-day-old rats, kept under culture conditions and then subjected to electrophysiological recording within 6-48 h. Records were taken at room temperature from a total number of 29 melanotropes. All of them showed spontaneous subthreshold fluctuations of membrane potential. These fluctuations were widely variable both in amplitude and duration from cell to cell. The average value for the resting potential was -73 ± 4 mV. In addition, 55% of the recorded cells spontaneously generated fast, spike-like depolarizations from the resting potential. These action potentials frequently overshot the 0 mV level by 26±5 mV (n = 9) and were triggered at an average rate of 1/min. In some cells, the repolarizing phase of the fast spike was followed by a plateau longer than 200 ms in duration. The average voltage level during the plateau was close to -15 mV. It will be important to investigate whether the onset of dopaminergic innervation modifies this pattern of electrical activity.

702.1

ONTOGENY OF FIRING PATTERNS OF DOPAMNE NEURONS IN SLICES.
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Bursts occurrence *in vivo* dopamine (DA) neurons is determined by N-Methyl-D-aspartate (NMDA) tone in midbrain DA areas, while DA neurons in slices exhibit only a regular pacemaker pattern. However, application of NMDA and other EEAs to slices from adult rats promptly increases the discharge frequency but fails to modify the pacemaker pattern of these neurons. In our study, DA neurons showed a biphasic, age-related, occurrence of bursting discharge. It increased from about 1% at post natal day (PND) 5 to a transient peak of more than 30% at PND 18, and rapidly decreased to zero after PND 45. In PND 15-21 slices, application of glutamate or kainate elevated the firing rate without pattern modification. On the contrary, NMDA (10-50 μM) activated discharge rate and also promoted burst firing in both bursting and non bursting neurons. NMDA induced bursts were age-related, maximal (37%) at PND 15-21, and NMDA increased the number of spikes in bursts irrespective of age. NMDA effects were not seen in a Ca^{2+} free solution or in the presence of NMDA antagonists. At PND 15-21, bath application of AP-5 reduced spontaneous burst occurrence and firing irregularity in 11 out 19 neurons. Whole-cell current-clamp recordings revealed spontaneous bursts in DA neurons at PND 15-21, superimposed on long (2-3 sec) depolarizing jumps. Low-threshold spikes, generating bursts of action potentials, were elicited by either depolarizing, (at -65 mV), or at the offset of hyperpolarizing currents steps (at -80 mV). Using electrodes filled with 145 mM CsCl, burst activity was paroxystically increased. In voltage-clamp recordings, spontaneous miniature excitatory post-synaptic currents (mEPSCs) were recorded in 8/12 bursting and in 1/10 pacemaker DA neurons at PND 15-21. Addition of 100 μM 7-Cl-KA abolished the mEPSCs and produced an outward current. Since burst firing modulates Ca^{2+} influx, thereby the Ca^{2+}-mediated DA release, the pharmacological manipulation of burst production might provide new strategies for those pathologies related to DA system dysfunction, including Parkinson's disease, abuse of drugs and schizophrenia. In addition, the elevated NMDA sensitivity displayed by DA neurons during the critical third week after birth might have relevance in NMDA-controlled phenomena, such as synaptic plasticity, neuro-architecture and neurotoxicity.

702.2

ASPHYXIA DURING BIRTH INDUCES LONG-LASTING CHANGES IN MESO-TELENCEPHALIC DOPAMINE RECEPTORS OF THE MALE RAT.
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Asphyxia was induced during birth to male Sprague-Dawley rat pups. At an age of 4 weeks dopamine receptors were analyzed by quantitative autoradiography in the mesencephalon (A9 and A10), the caudate nucleus, the accumbens nucleus, and the olfactory tubercle. Mild (15-16 min), as well as, severe (19-20 min) perinatal asphyxia reduced D_1 antagonist binding ([^{3}H]SCH-23390 in the presence of ketanserine) in the accumbens nucleus, the olfactory tubercle and the A9 region, and increased D_1 agonist affinity ([^{3}H]dopamine in the presence of raclopride) in the accumbens nucleus and the olfactory tubercle. Mild asphyxia did not change D_2 antagonist binding ([^{125}I] iodosulpride) or D_2 agonist affinity ([^{3}H]*N*-propylnorapomorphine), while severe asphyxia reduced D_2 agonist affinity in the accumbens nucleus. D_3 agonist affinity ([^{3}H]7-OH-DPAT) was increased only following mild asphyxia.

In conclusion, asphyxia during birth induces long-lasting changes in dopamine receptor binding in the meso-telencephalic dopamine systems, which may contribute to previously reported behavioral changes. Perinatal asphyxia may thus be of importance for development of neurodegenerative disorders.

702.3

DOPAMINE SYNTHESIS INHIBITION BY (±)-7-OH-DPAT IN STRIATUM, ACCUMBENS, AND PREFRONTAL CORTEX IN DEVELOPING RATS.
S. L. Andersen* and M. H. Teicher, Department of Psychiatry, Harvard Medical School, Laboratory of Developmental Psychopharmacology, McLean Hospital, Belmont, MA 02178.

Dopamine (DA) synthesis modulation by the D_3 receptor agonist (±)-7-OH-DPAT was explored in striatum, accumbens, and prefrontal cortex of developing rats (10 - 40 days of age) using the GBL autoreceptor model. GBL produced an age-dependent increase in DA synthesis that was inhibited by (±)-7-OH-DPAT (0.1 - 13.5 mg/kg) in all regions. In striatum 7-OH-DPAT exerted a greater maximal inhibitory effect at 10-20 days (85% inhibition) than at 30-40 days (61% inhibition). 7-OH-DPAT inhibited DA synthesis by 66% in the accumbens, with no significant change with age. In prefrontal cortex GBL increased synthesis between 10-30 days, but had no effect at 40 days. 7-OH-DPAT inhibited DA synthesis 60% between 10-30 days. Eticlopride antagonized the action of 7-OH-DPAT in all regions, suggesting DA receptor mediation. These data confirm the existence of synthesis-modulating autoreceptor function in prefrontal cortex that recedes by 40 days. In all regions, the IC_{50} increased with age, indicating a decrease in autoreceptor sensitivity with maturation. Curiously, the striatum was more sensitive to the inhibitory effects of 7-OH-DPAT than the mesolimbic regions. (See Table below; IC_{50} in mg/kg)

	Striatum	Accumbens	PrefrontalCortex
10 Days	<<0.1	0.27 ± 0.20	<<0.1
15 Days	0.49 ± 0.32	1.42 ± 0.46	0.79 ± 0.43
20 Days	0.41 ± 0.14	0.95 ± 0.46	2.97 ± 0.12
30 Days	0.44 ± 0.88	0.50 ± 0.09	3.71 ± 0.53
40 Days	2.46 ± 0.25	3.53 ± 0.64	-- -- --

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702.4

3.6 KILOBASES OF THE 5' FLANKING DNA OF THE MOUSE TYROSINE HYDROXYLASE GENE DIRECTS BRAIN-SPECIFIC BUT NOT CATECHOLAMINERGIC-SPECIFIC EXPRESSION.
W.W. Morgan*. Dept. of Cellular and Structural Biology, Univ. Texas Hlth. Sci Ctr. at San Antonio, TX 78284-7762.

The expression of the tyrosine hydroxylase (TH) gene is regulated by very precise molecular mechanisms which limit its activation to catecholaminergic neurons located within the brain, the sympathetic chain ganglia and paraganglia and to adrenal medullary cells. To study these processes, approximately 3.6 kilobases (kb) of the 5' flanking DNA of the mouse TH gene was inserted upstream of a E. coli β-galactosidase reporter (lac Z). This fusion gene (TH3.6LAC) was introduced into one-cell embryos of C57BL/6 mice, and transgenic mice were identified by PCR analysis. Analyses of β-galactosidase activity and the demonstration of TH3.6LAC mRNA by RNase protection assays suggested that the chimeric gene was activated in every region of the brain examined including the olfactory bulb, brainstem, hippocampus, cerebral cortex, striatum, cerebellum and diencephalon, as well as the adrenal gland. However, no evidence of TH3.6LAC activation was observed in the liver, kidney, spleen, lung or thymus. Similar results were observed in different transgenic founder lines. Histochemical analysis suggests that the reporter gene is activated in catecholaminergic and some selected non-catecholaminergic nuclei of the brain and in the adrenal medulla. Therefore, 3.6 kb of the 5' flanking DNA of the mouse TH gene appears sufficient to direct the expression of the lac Z reporter to the brain and the adrenal medulla but not to limit this activation to catecholaminergic nuclei. Supported by GM43763.

702.5

THE CREATION OF A TYROSINE HYDROXYLASE-NULL MUTATION.
M. Rios*, T. Sasaoka, D.M. Chikaraishi, and S. Roffler-Tarlov, Dept. of Anat. & Cell Biol.; Dept. of Neuroscience, Tufts. Univ., Boston, MA 02111

Cells containing the catecholamines dopamine, norepinephrine, and epinephrine are present in the central and peripheral nervous systems. Tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine biosynthesis, is persistently expressed by all catecholaminergic neurons and is transiently expressed in some cell populations during development. The role of TH in tissues where it is transiently expressed is not yet understood. The long term goal of the creation of a TH-null mutation is to expand our knowledge of catecholamine systems, transient TH expression, and the putative role of catecholamines in neural development.

Tyrosine hydroxylase-null mutants were generated using homologous recombination in embryonic stem (ES) cells. In order to disrupt the TH gene, a polyadenylation trap targeting vector was made in which genomic sequence between exons 6 and 8 of the mouse TH gene was replaced by a thymidine kinase-neomycin (TK-Neo) cassette without a polyadenylation signal. This construct was transfected by electroporation into D3 ES cells that were analyzed subsequently for the presence of homologous recombinants. Three homologous recombinant clones were detected among 371 neomycin-resistant clones, representing an efficiency of less than 1%.

Seven chimeras were generated from the two independent ES cell clones with ES cell contribution estimated to range from 65-100% based on coat color. Among the seven chimeras bred to C57Bl6 mice, five led to germ line transmission producing heterozygous carriers that were used subsequently to breed the mutation to homozygosity.

Lack of TH-null mutants in our first litters leads us to suspect that disruption of the TH gene results in embryonic lethality in homozygous animals, confirming the results of Zhou et al. (Nature, 1995, 374:640-643). NS20181 and NS22675

702.6

IN LAN-1 CELL LINE, TETRAHYDROBIOPTERINE INDUCES THE SYNTHESIS OF DOPAMINE AND REDUCES THE SEROTONIN TURNOVER.
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LAN-1 is a human cell line expressing tyrosine hydroxylase, muscarinic M1 and M3 receptors, veratridine-sensitive sodium channels and they lack voltage-sensitive calcium channel. To further characterize the aminergic phenotype of these cells, we measured the monoamine and metabolite content by HPLC, the high affinity uptake and the veratridine-induced release of [3H]dopamine. Immunocytochemical analysis using anti Tyrosine Hydroxylase (TH) antibodies confirmed that several cell were TH-positive: nevertheless, no dopamine (DA) nor DA metabolite (DOPAC and HVA) were found in the cell omogenate. However, significant amounts of serotonin (5HT) and its metabolite 5HIAA were measured in the cells (2.6 ±1.0 and 20.5 ±1.5 ng/mg protein at 1 d.i.v. and 3.0±0.7 and 19.3 ±2.2 ng/mg protein at 4 d.i.v, respectively). The presence in the culture medium of 5,6,7,8 tetrahydrobiopterine (THB, 10 mM), cofactor for both TH and tryptophane hydroxylase, was able to activate the dopamine syntesis: at 1 d.i.v, DA, DOPAC and HVA contents were 8.7 ± 2.7, 1.1 ±0.1 and 0.3 ± 0.1 respectively. Moreover, THB significantly decreased 5HIAA (24.9 ± 0.4 vs 8.1 ± 1.5 ng/mg protein in control and THB, respectively, $p<0.01$). Similar results were obtained in four-day old cultures. LAN 1 cells were also able to taken up exogenous DA. After incubation with 50 nM [3H]DA, the specific [3H]DA uptake was 456.7+17.7 fmol/well and 1231.0+33.0 fmol/well at 1 and 4 d.i.v. respectively. On the other hand, in [3H]DA preloaded cells, veratridine (100μM) was unable to induce dopamine release. Taken together these data indicate that LAN 1 cell express TH and the cellular machinery for the high affinity dopamine uptake: in these cells, the dopamine synthesis could be actvated by the presence of high concentrations of THB, making this cell line an useful model of human diseases such as dopa-responsive dystonia. THB is also able to reduce the serotonin turnover: this appears of particular interest for studing the role of serotonin-dopamine interactions during normal development and in the pathogenesis of human psychiatric disorders.

702.7

THE SEROTONERGIC SYSTEM EXHIBITS DISTINCT DEVELOPMENTAL PATTERNS IN CORTICAL AND SUBCORTICAL STRUCTURES. A. Dinopoulos[1], L. Dori[1] and J.G. Parnavelas[2] (SPONSORED BY ENA). Dept. Anat., Vet. Sch., Univ. Thessaloniki, Greece[1], Dept. Anat., University College London, U.K.

The serotonin (5-HT) innervation of the developing rat visual cortex (VC), lateral geniculate nucleus (LGN), superior colliculus (SC), lateral septum (LS) and basal forebrain (BF) was examined with light and electron microscope immunocytochemistry. Comparisons between these areas showed that: 1) the distribution pattern was established gradually in some areas (LGN, SC, LS, BF), whereas in others (VC) it showed transient features; the adult pattern of innervation was attained by the end of the third postnatal week in all areas; 2) the types of synapses formed by 5-HT varicosities and the nature of postsynaptic profiles varied according to age and area of the brain examined; and 3) in all areas, the proportion of labelled varicosities forming synapses increased from birth until the end of the second week, declined markedly in the subsequent week before increasing again at a later stage. These findings support the view that: 1) the organization of the 5-HT system is related to the postsynaptic targets and, although arising in a small number of neurons in the midbrain, is highly specific in many parts of the brain; and 2) synapses formed by the serotonergic system during the first two weeks of life may be related to the involvement of 5-HT in developmental events, while those formed after the third week of life may be involved in the mediation of neurotransmitter effects by this monoamine.

702.8

ANALYSIS OF THE EXPRESSION AND LOCALIZATION OF GLYCINE RECEPTOR ALPHA2 SUBUNITS IN RAT SPINAL CORD WITH A POLYCLONAL PEPTIDE ANTIBODY. M.V. Langston and P.A. St. John.* Dept. of Cell Biology & Anatomy, Univ. of Arizona, Tucson, AZ. 85724.

Glycine receptors (GlyRs) are pentameric proteins composed of alpha and beta subunits. Molecular biological techniques have demonstrated the existence of multiple subtypes of alpha subunits and have shown that mRNA for alpha2 subunits is present in prenatal rat spinal cord. Immunological assays also have suggested that GlyR alpha2 subunits are present during early developmental stages in the rat spinal cord. Direct demonstration of the presence and specific localization of alpha2 polypeptides has been lacking, however, due to the absence of an antibody specific for this protein. We have raised a polyclonal antibody against a 10-amino acid peptide sequence, specific to the rat GlyR alpha2 subunit. Using this antibody, we have performed experiments on different ages of rat spinal cord neurons *in vivo* and *in vitro* to examine the expression of the GlyR alpha2 subunit. Analysis by Western blots showed that expression of the GlyR alpha2 subunit differed among the various developmental stages of rat spinal cord neurons, both *in vivo* and *in vitro*, as expected from previous results. In addition, confocal microscopy indicated that the GlyR alpha2 subunits are present on neuronal surfaces as early as embryonic day 14. In prenatal tissue, labeling was seen on most or all neurons, while in adult tissue labeling was predominantly in motoneurons. Clustering of GlyRs appeared to be much less striking than that previously demonstrated in adult rat spinal cord. We conclude that the GlyR alpha2 subunit is expressed on the surfaces of prenatal rat spinal cord neurons early in development *in vivo* and at a comparably early time *in vitro*. (Supported by NINDS #NS29657).

702.9

POSSIBLE POPULATION DIFFERENCES IN THE EXPRESSION OF GLYCINE RECEPTOR α2 ISOFORMS BY EMBRYONIC RAT SPINAL CORD NEURONS DURING DEVELOPMENT. M. D. Withers* and P. A. St. John. Program in Neuroscience, Dept. of Anatomy, University of Arizona, Tucson, AZ 85724.

Previous results radio-ligand binding assays, fluorescence microscopy with monoclonal antibodies and whole-cell patch-clamp recordings provide evidence for a change in the predominant expression of GlyR α subunits by embryonic rat spinal cord neurons from a relatively strychnine-insensitive isoform, presumed to be α2*, to one or more strychnine-sensitive isoforms one of which is shown to be α1. Recent experiments employing these same three techniques on embryonic spinal cord neurons from a different population of rats provide evidence for the predominant expression of strychnine-sensitive GlyR isoforms at all times tested. [3H]-strychnine binding is high on spinal cords and cultured spinal cord neurons from E14 rats while previous experiments on the original rat population showed no detectable [3H]-strychnine binding sites on E14 rat spinal cords or on neurons from these cords before four days in culture. Responses to glycine by spinal cord neurons from the recent population of rats are also completely blocked by 10 uM strychnine, while this concentration of strychnine blocked responses to glycine by same-aged neurons from the previous rat population by only 20 percent. Polyclonal antibody experiments provide evidence for the expression of a GlyR α2 subunit at early times in culture. These results provide evidence for the expression of the strychnine-sensitive α2 isoform of the GlyR by embryonic spinal cord neurons from the recent rat population at a time when a different population showed expression of a strychnine-insensitive isoform of the GlyR. (Supported by NIH and The Robert S. Flinn Foundation).

702.10

HIGH-AFFINITY ADENOSINE A2 RECEPTOR MATURATION IN THE RAT STRIATUM AND THEIR COUPLING TO G PROTEINS. J.L. Daval*, J.F. Doriat and A.C. Humbert. INSERM U.272, 30 rue Lionnois, 54013 NANCY, FRANCE.

To study the cerebral development of adenosine A2a receptors, the specific binding of [^{3}H]CGS 21680, a selective agonist, was measured in striatal membranes as well as in brain sections from the rat between birth and adulthood. The distribution of [^{3}H]CGS 21680 binding sites was analyzed by autoradiography, and the functional coupling of A2a receptors to G proteins was studied by the addition of a stable analogue of GTP, i.e. Gpp(NH)p. The binding of [^{3}H]CGS 21680 (0.2-80 nM) was assessed during a 90 min incubation in Tris-HCl (50 mM, pH 7.4) containing 10 mM MgCl2 and 2 IU/ml adenosine deaminase in the absence or in the presence of 10 µM Gpp(NH)p. In the striatum of adult rats, the total number of binding sites for [^{3}H]CGS 21680 reached 425±55 fmol/mg prot, with a Kd of 15.3±1.5 nM. A2a receptors were concentrated in caudate and accumbens nuclei, olfactory tubercles and globus pallidus. Such a specific distribution was observed at all developmental stages. At birth, the density of receptors was low but detectable, around 3% of the adult value, and receptor affinity was 8-fold higher than in adult (Kd=2.1±0.4 nM). The number of binding sites for [^{3}H]CGS 21680 increased with postnatal age to reach 16% of adult value at 5 days, 30% at 10 and 15 days, and 69% at 25 days, respectively. Concomitantly, Kd increased during brain development (4.5 nM at 5 days, 6.0 nM at 10 and 15 days, 9.8 nM at 25 days). Gpp(NH)p reduced significantly Kd values at all ages (from 47 to 82%), showing that receptors are linked to transducing proteins. Highest affinities of A2a receptors in the neonatal period may reflect a specific role for these sites in the immature brain, and A2a ontogeny contrasts with the developmental pattern of A1 receptors.

702.11

Differential Regulation Of Adrenergic Receptor Development By Sympathetic Innervation. Beth A. Habecker*, Nell M. Malec, and Story C. Landis. Dept. of Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

Alpha and beta adrenergic receptors (α and β AR) mediate the effects of the sympathetic nervous system in peripheral tissues. Sweat glands are an unusual sympathetic target, since activation of AR during early postnatal development is critical for a target-directed switch of transmitter properties in the sympathetic innervation, while the mature innervation elicits sweat secretion through cholinergic stimulation of muscarinic receptors. Sweat glands are an interesting system for the study of regulation of AR expression because by postnatal day 21 catecholamines, which alter AR expression in many in vitro studies of receptor regulation, are gone and the sympathetic innervation contains acetylcholine, vasoactive intestinal peptide (VIP), and calcitonin-gene-related peptide (CGRP). Alpha 1B and β2 receptors are present in rat sweat glands; both receptor subtypes decrease after P21. This timing raises the possibility that changes in the sympathetic innervation influence receptor expression. Neonatal sympathectomy causes a partial failure of α1B downregulation, but has no effect on β2 levels. Therefore, innervation-independent processes control developmental expression of α1 and β2, while an additional innervation-dependent mechanism contributes to regulation of α1 receptors. Denervation at postnatal day 20, when the sympathetic innervation is cholinergic and peptidergic, leads to retention of adult α1 receptors similar to that seen with neonatal sympathectomy, but cholinergic blockade begun at P20 does not. These findings suggest that a heterologous mechanism, possibly utilizing VIP or CGRP, mediates the innervation-dependent portion of the developmental decrease in α1 receptors. Thus, multiple mechanisms, some utilizing the sympathetic innervation, control adrenergic receptor levels during sweat gland development. This work supported by NS23678 and NS09336.

702.12

HEME OXYGENASE ACTIVITY IN THE GUINEA PIG HIPPOCAMPUS, CEREBRAL CORTEX, AND CEREBELLUM: ONTOGENY AND ETHANOL EXPOSURE. MN Cook[†], HJ Vreman[‡], GS Marks[†], K Nakatsu[†], DK Stevenson[‡], & JF Brien[†]* [†]Dept. Pharmacology & Toxicology, Queen's Univ., Kingston, Canada, K7L 3N6. [‡]Dept. Pediatrics, Stanford Univ., Stanford, CA 94305.

Heme oxygenase (HO) catalyzes the oxidation of heme to carbon monoxide (CO) and biliverdin. HO appears to be involved in cell-cell communication via its regulation of CO, a novel neuronal messenger, and heme, a key constituent of nitric oxide synthase and soluble guanylyl cyclase. Ethanol CNS teratogenesis, a principal feature of the fetal alcohol syndrome, may involve altered function of HO during brain development. The ontogeny of HO activity in the hippocampus (H), frontal cerebral cortex (CC), and cerebellum (C) of the guinea pig at selected prenatal and postnatal ages and the effect of *in vitro* ethanol exposure were determined. Fetal guinea pigs at gestational day (GD) 51 and GD 63 (term, about GD 68), and adult guinea pigs were studied. The microsomal fraction of each brain region was analyzed for HO activity by an optimized method which measures the NADPH-dependent formation of CO from 25 µM methemalbumin at 37°C using a gas chromatographic method to quantitate CO. For each brain region, HO activity was greatest ($p < 0.05$) in the GD 63 fetus compared with the GD 51 fetus and the adult. There was no difference in HO activity at each developmental age among the three brain regions. *In vitro* ethanol (25-100 mM) exposure of microsomes did not affect HO activity. The data demonstrate, for the guinea pig, a distinct ontogenic profile for HO activity in the H, CC, and C. The effect of chronic prenatal ethanol exposure on HO activity in these brain regions is being studied. (Supported by the Medical Research Council of Canada).

702.13

EXPRESSION OF CALCITONIN GENE-RELATED PEPTIDE IN AXOTOMIZED RUBROSPINAL NEURONS

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Axon injury induces an up-regulation in the synthesis of calcitonin gene-related peptide (CGRP), as well as tubulin and GAP-43 in spinal, facial, hypogrossal motoneurons. The rubrospinal neurons, that are confined within the CNS, have also been shown to increase the expression of tubulin and GAP-43 following axotomy. This study was designed to examine whether CGRP expression is induced in axotomized neurons in the rat red nucleus.

The cervical spinal cord of male Sprague-Dawley rats (140-200g) was exposed between C1-C2 lamina arcus vertebrae, and the left lateral funiculus was transected. Seven days after spinal hemisection, animals were killed by decapitation, and the brains were processed for *in situ* hybridization with radiolabeled oligonucleotide CGRP probe. Another group of animals were killed by paraformaldehyde perfusion 14 days after spinal hemisection. The change in CGRP-like immunoreactivity (LI) in red nuclei was examined using immunohistochemistry.

In rubral neurons contralateral to the spinal hemisection, we could detect neurons labeled for CGRP mRNA. There was no rubral neurons expressing CGRP mRNA on the ipsilateral side. Immunohistochemistry showed that the axotomized rubral neurons express increased CGRP-LI compared to the contralateral neurons. These data indicate that axotomy induces an up-regulation of CGRP synthesis in rubrospinal neurons, suggesting that neurons in CNS may be capable of synthesizing a neuropeptide *de novo* in response to the axonal injury.

702.14

DEVELOPMENT OF THE RAT BRAIN HISTAMINERGIC SYSTEM IN RELATION TO THE SEROTONERGIC AND CATECHOLAMINERGIC SYSTEMS AND PROGRAMMED CELL DEATH.

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In order to test the apparent spatio-temporal similarities in the distribution of the different developing aminergic systems implicated by separate earlier studies, the distribution of histamine-immunoreactive (HA-ir) neurons and nerve fibers was compared to the distribution of 5-hydroxytryptamine- (5-HT) and tyrosine hydroxylase-immunoreactive (TH-ir) ones in the developing Wistar -rat brain between embryonic days 12 (E12) and 20 (E20) using a double immunostaining method. The authentity of the immunohistochemically detected histamine of the developing brain was verified using high-pressure liquid chromatography (HPLC) fluorometric method. Synthetic oligonucleotide probes complementary to the rat histidine decarboxylase (HDC) were used for determination of the origin of HA in the developing brain by *in situ* hybridization.The immunohistochemical results revealed a transient co-localization of HA and 5-HT between E14 and E18 within a subgroup of cells in the developing raphe nuclei where also a positive HDC- hybridization signal was detected at E16. From E18 onwards the HA-ir neurons started to disappear gradually from the rhombencephalon and could no more be seen at E20. No significant co-localization of HA and TH was detected. The fate of the transiently HA-ir neurons was studied further using an *in situ* apoptosis detection kit for DNA fragmentation. The preliminary results showed a positive hybridization signal at E18 in the ventral pons rendering it possible that the "dissappearing" HA-ir neurons with the same location could die apoptotically. As no migrating HA-ir neurons were detected , an equally possible explanation is the change in the phenotype of the HA-ir cells following the cessation of histamine synthesis in the rhombencephalon. *The study presented in this abstract was the 1994 winner of the GBWest Memorial Competition.*

702.15

EXCITATION-SECRETION COUPLING IN CHOLINERGIC NERVES WITHIN THE EMBRYONIC CHICK HEART.

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In embryonic chick atria, potassium-evoked secretion of acetylcholine (ACh) from vagal and intramural parasympathetic neurons is calcium dependent and blocked by dyhydropyridines (Gray et al., Soc. Neurosci. Abstr., 1994). Incubation of atria with conotoxins such as ωGVIA or ωMVIIC at umolar levels had little effect on evoked ACh release at 14 days *in ovo*, but both toxins blocked over 90% of potassium-evoked ACh release at hatching. These data support the hypothesis that calcium channels coupled to transmitter release change from primarily L type to N type over hatching as in another parasympathetic neurons in the avian ciliary ganglion. To confirm this hypothesis for stimulation, ACh release was evoked from isolated atria with field electrical stimulation from platinum electrodes. Electrodes were connected to two grass stimulators and suspended in microfuge tubes containing freshly isolated atria. Stimuli were administered at a pulse frequency of 10 Hz, pulse duration of 20 msec, and stimulation period of two min. Labeled ACh release evoked by field stimulation was approximately 4 times higher than background and was calcium dependent. Incubation with 10 um nifedipine significantly inhibited evoked ACh release from all atrial preparations: release from continous stimulation was inhibited by 85%, while patterned stimulation using pulse trains was inhibited by 65%. Although further pharmacological analysis will test this possibilty it is clear that L type calcium channels do play a prominent role in transmitter release in the embryonic heart. Supported by The Catherine and Patrick Donaghue Foundation for Biomedical Research.

702.16

DEVELOPMENT OF CHOLINERGIC RECEPTORS IN FOREBRAINS OF RATS WITH 192 IgG-SAPORIN INDUCED CHOLINERGIC DEFICITS.

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Previous work has demonstrated that an immunotoxin, consisting of the 192 IgG antibody to the low affinity NGF receptor coupled to the ribosome inactivating protein saporin, leads to the loss of basal forebrain cholinergic neurons. This project is continuing to examine the effect of early destruction of basal forebrain cholinergic neurons on development of cholinergic receptors in postnatal rat cerebral cortex and hippocampus. Injections of 192 IgG-saporin were made intraventricularly in Sprague-Dawley rat pups on postnatal day 0 (P0) and again on P2, At P8 or P21, animals were sacrificed and forebrains were processed for receptor autoradiography. AChE histochemistry and hemicholinium uptake were used to verify extent of the cholinergic lesion. Results from studies of hippocampus indicate a marked up-regulation of some forms of muscarinic receptors, but not nicotinic receptors. Patterns of M1 and M2 receptor populations, as indicated by pirenzipine and AFDX binding, appear no different in saporin treated and control animals. Binding of NMS, in the presence of M1 and M2 blockers, indicates a marked up-regulation of non-M1 and non-M2 receptors in hippocampus. Up-regulation was most prominent in the dentate gyrus, moderate in CA1 and CA2, and least in CA3. No changes in muscarinic receptor binding were apparent in neighboring cingulate and somatosensory cortical areas. These data indicate that the loss of forebrain cholinergic neurons produced by 192 IgG-saporin results in a selective alteration of cholinergic receptors. *Supported by NIH grant NS 30109 and the Department of Veterans Affairs.*

702.17

NEUROCHEMICAL PHENOTYPES OF POSTNATAL RAT MYENTERIC NEURONS IN DISSOCIATED CELL CULTURE

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We have recently developed new methods for growing myenteric ganglia from the postnatal rat small intestine in dissociated cell culture. These cultures contain neurons, which can be identified by immunolabelling with antiserum to protein gene product 9.5 and glia, which can be identified by immunolabelling with antisera raised against S100. We have previously shown that both basic fibroblast growth factor and the adenosine analogue, 2-chloroadenosine have trophic actions on neurons in these cultures[1]. However, different subpopulations of enteric neurons were not investigated in this earlier work. In order to assess the suitability of this model for the study of the actions of trophic factors on identified populations of postnatal myenteric neurons, we have now studied the expression of NADPH diaphorase activity and neuropeptides in these cultures. Myenteric ganglia were separated from the muscularis externa of the small intestines of 7 - 10 day old rats by mechanical agitation of small pieces of tissue which had been treated with collagenase. The isolated ganglia were then dissociated in trypsin, seeded on to poly-L-lysine coated coverslips and grown in a serum-free culture medium. After 2-5 days in culture the cells were fixed in 4% paraformaldehyde and processed for the histochemical reaction for NADPH diaphorase activity or immunolabelled using antisera raised against vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and methionine enkephalin (mENK). Populations of neurons in these cultures were found to express NADPH diaphorase activity or VIP, NPY and mENK immunoreactivity. These observations indicate that such cultures will be a useful model for the study of the effects of neurotrophic factors on identified populations of postnatal myenteric neurons.

1. K-H.Schafer, MJ. Saffrey and G.Burnstock (1995) Neuroreport 6 (6).

702.18

PRENATAL DEVELOPMENT OF NITRIC OXIDE SYNTHASE IN THE MOUSE SPINAL CORD.

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The prenatal expression of neuronal nitric oxide synthase in the mouse spinal cord was investigated by NADPH diaphorase histochemistry and immunocytochemistry. Around embryonic day (E) 12, the first positive neurons were located in the intermediolateral position of the thoracic and sacral spinal cord. Two days later, presumed sympathetic cells were also located in an intermediomedial position. Further, positive neurons were grouped around the ventricular zone in the ventral part of the spinal cord. Between E15 and the first postnatal days, a group of cells in the ventral horn of the cervical and lumbar cord transiently stained for NADPH diaphorase. However, attempts to stain this subpopulation of ventral horn cells by immunocytochemistry failed.

Comparison with published data on the ontogenetic expression pattern of choline acetyltransferase and GABA indicates that expression of nitric oxide synthase starts after the cholinergic and/or GABAergic phenotype is acquired. Regulatory functions of nitric oxide in the development of the mouse spinal cord appear to be confined to later stages of neuronal migration and differentiation.

703.1

CHARACTERIZATION OF PC12 CELLS WHICH OVER- AND UNDEREXPRESS S100A1. E. H. Cornwall*, P. D. Reynolds, C. M. Donald and D. B. Zimmer. Department of Pharmacology, College of Medicine, University of South Alabama, Mobile, AL 36688.

S100A1 is a small, acidic calcium-binding protein, which is expressed in a variety of tissues and cells, including neurons. S100A1 modulates a number of intracellular processes via interaction with target proteins such as glycogen phosphorylase, aldolase and tau protein. Furthermore, documented increases in S100A1 levels appear to be associated with unregulated cell growth. To determine what cellular processes are regulated by S100A1, stable transfectants of PC12 cells which over- and underexpress S100A1 have been prepared using a mammalian expression vector which contains the S100A1 cDNA in the sense (pSense) or antisense (pAntisense) orientation with respect to a cytomegalovirus promoter. These DNA constructs were transfected into PC12 cells using electroporation. Reverse transcriptase-polymerase chain reaction analysis demonstrated that three clones expressed the pSense mRNA and three clones expressed the pAntisense mRNA. Indirect immunofluorescence microscopy showed that two clones which expressed the pSense mRNA had a greater S100A1 antibody fluorescence staining when compared to control cells. Three clones which expressed the pAntisense mRNA had decreased fluorescence staining when compared to control cells. These results suggest that the expression of the pSense and pAntisense mRNAs resulted in increased and decreased S100A1 levels, respectively, in PC12 cells. Functional studies on these clones will reveal which target proteins are regulated by S100A1. Furthermore, these studies will provide insights into the consequences of altered S100A1 expression and its role in disease progression. This research is supported by a grant from the NIH (30660).

703.2

ACTIVIN TYPE IIA RECEPTOR mRNA EXPRESSION BY NEURONS OF THE AVIAN CILIARY GANGLION. K. Kos, and J.N. Coulombe* Dept. of Anatomy and Cell Biology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

Previous studies in culture have suggested that the protein activin may serve as a neurodifferentiation factor regulating the expression of somatostatin in neurons of the avian ciliary ganglion. We therefore sought to determine if any of the known activin receptors (ActR) are expressed by developing ciliary ganglion neurons in vivo.

Oligonucleotide primers were designed for the chicken ActR type IIA and type IIB. Both of these primer sets amplified bands of the anticipated sizes in reverse transcription polymerase chain reactions (rtPCR) with target RNA isolated from chicken ovaries. When RNA isolated from ciliary ganglia was used as the target instead, only primers corresponding to the ActR type IIA resulted in rtPCR amplification of an appropriately sized product. This rtPCR product was subcloned and sequenced, which confirmed it's identity as a fragment of the chicken ActR type IIA. It thus appears that mRNA for the type IIA but not the type IIB ActR is expressed in the chicken ciliary ganglion.

To examine what cell types contain mRNA for this ActR, digoxigenin-labeled riboprobes from the cloned ActR type IIA fragment were used for in situ hybridization on cryostat sections of embryonic chicken ciliary ganglia. Antisense strand, but not sense strand, ActR type IIA specific riboprobes hybridized to cells within these sections. At the ages examined (E6-E17), the hybridization of these probes appeared to be specific for cells with a neuronal morphology. These results are consistent with the hypothesized role for activin as a neurodifferentiation factor in vivo, and imply that, within the ciliary ganglion, the ActR type IIA is specifically expressed by neurons. (Supported by NSF IBN 9309932, USUHS RO70DU)

703.3

EXPRESSION OF FGFR ISOFORMS IN THE DEVELOPING MURINE NERVOUS SYSTEM

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Acidic Fibroblast growth factor (FGF-1) and basic FGF (FGF-2) have potent effects on the proliferation and differentiation of neural precursor cells. FGFs mediate biological activity through tyrosine kinase cell surface receptors known as the fibroblast growth factor receptors (FGFRs). A family of four, highly homologous FGFR genes (FGFR1-4) have been identified. Specific sequences encoded by separate exons in the FGFR gene are alternatively spliced into the C-terminal half of loop III to create various isoforms that have different binding affinities for the FGFs. There is evidence that FGF action is regulated by spatiotemporal differences in the expression of these FGFR isoforms. We have designed an RT-PCR assay to both detect expression of the various isoforms of the FGFRs in the embryonic nervous system and to generate isoform specific cRNA probes for use in RNase protection and *in situ* hybridization analyses. An RT-PCR assay on mRNA from embryonic day 10 (E10) neuroepithelium has shown that it expresses isoforms of FGFR1, 2 and 3, but not of FGFR4. RT-PCR and RNase protection analyses on mRNA from an E10 neuroepithelium derived cell line (2.3D) demonstrated that the expression of FGFR1 IIIac is very high compared to the expression of other isoforms. The FGFR1 IIIac isoform seems to be the only isoform in this cell line, which is used to poentiate the action of FGF-2 (1). The distribution of FGFR protein is currently beeing investigated using antibodies specific to FGFR1, 2 and 3.

1. Brickman, Y. et al., 1995, J.Biol.Chem. (subm)

703.4

FGF RECEPTOR IS A NUCLEAR PROTEIN - NOVEL MECHANISM FOR bFGF ACTION. **E.K. Stachowiak, E. Mordechai, A. Joy, , A. Schwartz*, P. Maher*, M.K. Stachowiak. Barrow Neurol.Inst. Phoenix, Az 85013, *The Scripps Res.Inst. La Jolla, CA 92037.**

Traditionally growth factors (GFs) are thought to exert their biological effects by interacting with plasma membrane receptors. However, some GFs including bFGF, aFGF, or CNTF lack secretory signal sequences and are primarily cell associated. Trans-synaptic or hormonal stimulation of adenylate cyclase in bovine adrenal medullary cells (BAMC) leads to nuclear accumulation of bFGF suggesting a direct nuclear function of bFGF (Stachowiak et al., 1994). To determine how nuclear bFGF could exerts its biological effects we examined the subcellular distribution of its receptors (FGFR). Immunoprecipitation with antibodies against different types of FGFR showed that FGFR1 is the only type expressed in cultured BAMC. Confocal microscopy and Western analyses of fractionated BAMC demonstrated that FGFR1 protein is located predominantly in the nucleus, with little cytoplasmic or plasma membrane localization. Nuclear FGFR1 is represented by 103 kDa protein containing both the N-terminal (ligand binding) and C-terminal (tyrosine kinase, TK) domains, and by 118 kDa and 145 kDa proteins recognized only with the N-terminal FGFR1 antibody. Consistently with these results we detected high affinity ^{125}I-bFGF-binding sites (kd=14 pM) and bFGF-stimulated TK activity in nuclear extracts. Similarly as bFGF, the FGFR1 colocalize with the nuclear matrix and nucleoplasm, but not with chromatin. Electron microscopy revealed patches of FGFR1 colocalized with bFGF patches within the interior of the nucleus. Treatment of BAMC with forskolin induced parallel accumulation of bFGF, FGFR1 and bFGF-TK in the nucleus. Nuclear translocation of bFGF and its receptors offers a novel mechanism for GF action (Supported by NIH, NSF, APDA).

703.5

CHARACTERIZATION OF LIF-RECEPTOR (LIF-R) ANTAGONISTS IN BIOLOGICAL ASSAYS. A.B. Vernallis*, K. Hudson, and J.K. Heath. Dept. of Biochemistry, Oxford University, Oxford, OX1 3QU, U.K.

LIF-R and gp130 oligomerize and activate intracellular signals in response to LIF, OSM, CNTF/CNTFR, and CT-1. Given the large number of ligands, antagonists of LIF-R function would be useful in probing the role of LIF-R.

As a first approach, residues on the A-helix of human LIF, that are analogous to site II of human growth hormone based on the crystal structures of murine and human LIF, were substituted with alanine to generate a LIF mutant with reduced ability to interact with gp130. The resulting mutant binds LIF-R with normal affinity but has a much lower affinity for gp130. In proliferation assays on Ba/F3 cells cotransfected with hLIFR and hgp130, the mutant inhibited 50% of the proliferation response to a submaximal dose of hLIF at a 30-100-fold excess of competitor. Responses to OSM were also reduced.

Second, in order to generate LIF-R that binds ligands but does not signal, the extracellular domain of hLIFR was truncated after the membrane-proximal hematopoietin domain and fused to the Fc region of human IgG1. This soluble receptor (LIFR-Fc) was expressed in 293T cells and purified on protein A. In Ba/F3 assays the proliferation response to hLIF was reduced by 50% at approximately a 1000-fold molar excess of LIFR-Fc.

Preliminary results suggest that both these antagonists reduce the hLIF stimulation of the VIP promoter in IMR-32 neuroblastoma cells indicating that the antagonists will be useful in studying the function of LIF-R in the nervous system.

703.6

VIP ANALOGUE STIMULATES EMBRYONIC GROWTH THROUGH GTP-INSENSITIVE BINDING SITES. I.M. Hill*, D.A. Dibbern Jr., I. Gozes, M. Fridkin and D.E. Brenneman. Lab. of Dev. Neurobiol. NICHD, NIH, Bethesda, MD 20892; Dept. of Clinical Biochem. Tel Aviv Univ., Tel Aviv, Israel; Dept. of Organic Chem. Weizmann Inst. of Science, Rehovot, Israel.

Vasoactive intestinal peptide (VIP) has been shown to act through GTP-insensitive binding sites to regulate embryonic growth during the early post-implantation period (*Nature*, 1993, 362:155; *J. Clin. Invest*. 1994, 94:2020). Stearyl-norleucine-VIP (SNV), a novel VIP analogue which acts via cAMP-independent mechanisms, has recently been shown to enhance neuronal survival (*J. Pharm. Exper. Ther*. 1995, 273). The purpose of the current study was: 1) to examine the action of SNV on the growth of cultured day 9.5 mouse embryos; and 2) to compare the distribution of SNV binding sites in CNS with GTP-insensitive VIP binding sites. SNV and GTP-insensitive binding sites were determined by *in vitro* autoradiography with ^{125}I-VIP in the presence of 10^{-6}M SNV or 10^{-5}M GMP-PNP (a stable GTP analogue), respectively, on adjacent sections. Treatment of mouse embryos in culture with 10^{-7}M SNV resulted in a significant increase in growth. SNV-treated embryos grew 3.7 somites during the four hour incubation period compared with 2.2 somites by controls and 5.2 somites by 10^{-7}M VIP. Embryonic growth was not stimulated by treatment with the related peptide PACAP (10^{-9}M-10^{-7}M). *In vitro* autoradiography revealed that SNV displaced ^{125}I-VIP only at specific sites in the CNS and those sites coincided with GTP-insensitive VIP binding sites identified on adjacent sections. Co-treatment with both GTP and SNV resulted in the total displacement of radiolabeled VIP from brain sections. These data suggest that VIP-induced stimulation of embryonic growth is regulated, in part, through cAMP-independent mechanisms and that SNV acts on GTP-insensitive binding sites in the brain.

703.7

ALK-7, A NOVEL BRAIN SPECIFIC SERINE-THREONINE KINASE RECEPTOR. M.Rydén, H.H. Jörnvall, M. Trupp and C.F. Ibáñez*
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Receptors for members of the transforming growth factor beta (TGF-β)-superfamily have been classified in three types, from which only type I and II represent signalling receptors with intrinsic serine-threonine kinase activity. To isolate new members of this receptor family specific for the nervous system, PCR was performed using primers derived from conserved regions of ser-thr kinase domains using rat P1 brain cDNA. Different fragments showing similarities to both type-I and type-II receptors were found, one of these corresponded to a previously nondescribed type-I ser-thr-kinase receptor which we have named ALK-7. A full-length cDNA clone was isolated and shown to contain a 494 aa open reading frame displaying all the characteristic functional domains of type I receptors. The amino-acid sequence of ALK-7 shows up to 66% identity to ALK-4 and ALK-5. RNAse protection assays and *in situ* hybridization demonstrated a highly specific pattern of expression restricted to the central nervous system. ALK-7 mRNA was predominantly expressed in postnatal cerebellum and hippocampus. *In vitro* translation generated a major 55 kDa product, in accordance with the predicted aa sequence. Ectopic expression in COS cells and ^{125}I-surface labeling showed ALK-7 to be a surface protein. Receptor reconstitution and cross-linking experiments are currently being performed to determine whether any of the known members of the TGF-β ligand superfamily, including TGF-β1, TGF-β2, activin, bone morphogenetic proteins (BMPs) and glial cell line-derived neurotrophic factor (GDNF), is a ligand for ALK-7.

703.8

A comparative study of two splice variants of the "neu"-regulin family.

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Proteins encoded by the neuregulin gene are comprised of multiple structural and functional domains. Alternative splicing of neuregulin gene transcripts result in over twenty protein isoforms. Some isoforms are predicted to result in proteins with similar structural domains with different sequences. Work by Wen et al has demonstrated that sequence differences between the α and β isoforms of the EGF-like domain result in proteins with different affinities for the receptor. Other splice variants produce isoforms with significantly different predicted structures. These differences in protein structure are likely to translate into differences in functional properties.

We have cloned a novel splice variant of the neuregulin family which lacks an immunoglobulin-like domain in its N-terminus (called nARIA). Instead, the nARIA splice variant has a cysteine rich domain at its N-terminus. Furthermore, the predicted amino acid sequence suggests that nARIA also possesses a signal sequence, a structural motif present in only one other reported isoform (GGF). The distribution of the nARIA message is also different from that of the otherwise similar immunoglobulin-containing isoform, ARIA. To study the functional differences of nARIA and ARIA, we have artificially manipulated the expression and structure of these genes. (Supported by NS29071)

703.9

ANALYSIS OF MUTANT PDGF RECEPTORS EXPRESSED IN PC12 CELLS IDENTIFIES SIGNALS GOVERNING NA CHANNEL INDUCTION DURING NEURONAL DIFFERENTIATION G.R. Fanger[1]*, R.R. Vaillancourt[3], L.E. Heasley[4], J.P.R. Montmayeur[3], G.L. Johnson[3,4] and R.A. Maue[1,2] Departments of [1]Biochemistry and [2]Physiology, Dartmouth Medical School, Hanover, NH 03755, [3]Divison of Basic Sciences, National Jewish Center for Immunology and Respiratory Medicine, and [4]Deptartment of Pharmacology, Univ. of Colorado Medical School, Denver, CO 80262

Although growth factors that stimulate tyrosine kinase receptors play an important role in neuronal differentiation, the mechanisms by which this occurs are not well understood. To identify signals necessary for the induction of voltage-dependent Na channel expression, a crucial aspect of neuronal differentiation that can occur independent of p21/ras activation, patch clamp analysis and RNase protection assays were used to analyze PC12 cells stably expressing platelet-derived growth factor (PDGF) receptors with mutations in tyrosine residues of the cytoplasmic domain that associate with signaling molecules upon activation of the receptor. Mutations that block activation of PI3-K, PLCγ, GAP, and Syp did not alter the PDGF-mediated induction of Na channel expression. However, mutation of tyrosines that associate with members of the src family of kinases significantly inhibited induction of functional Na channel expression in response to PDGF. Furthermore, mutations that block association with src family members, in combination with mutations that block PI3-K, PLCγ, GAP and Syp activation, abrogated the PDGF-mediated induction of type II Na channel α subunit mRNA, indicating that multiple signals generated by the receptor contribute to this response. The results were due to the specific mutations introduced and not simply the result of an overall decrease in receptor kinase activity, since transin and c-fos mRNA were still induced by all of the mutant receptors analyzed. The results provide insight into the signal transduction mechanisms governing expression of type II Na channel α subunit mRNA and Na current density, and mechanisms underlying neuronal differentiation. Supported by NS28767 to RAM.

703.10

PREGNENOLONE SULFATE INCREASES THE GROWTH OF HIPPOCAMPAL NEURONS. R. D. Brinton* and R. Gunawan. Dept. Molecular Pharmacology and Toxicology, University of Southern California, Los Angeles, CA 90033

Pregnenolone sulfate (PS) has been found to be synthesized de novo in oligodendrocytes of the hippocampus and cortex thereby providing locally synthesized concentrations of this neurosteroid. PS has also been found to enhance memory functions in mice and rats. Growth in the projections and synaptic connections has been postulated to be one of the fundamental cellular mechanisms involved in learning and memory. We therefore conducted experiments to determine the impact of PS on the growth and morphology of hippocampal nerve cells. Results of this investigation demonstrated that PS induced a significant increase in the number of neurites and the total length of neurites (*p<.05, ** p<.01, ***p<.001). Significant increases also occurred in the fine structure of the nerve cells. PS induced a significant increase in the number of branches (*p <0.05, *** p<.001), in the total branch length (*p <0.05, *** p<.001), in the number of bifurcation points emanating from neurites (*p <0.05, *** p<.001) and in the number of microspikes (*p<.05, ** p<.01, ***p<.001). We pursued the mechanism of PS-induced nerve cell growth by investigating the impact of blocking the NMDA glutamate receptor on PS-induced hippocampal nerve cell growth. Preliminary results of these experiments indicate that the growth promoting effects of PS (500 nM ** p<.01) are completely abolished in the presence of the NMDA receptor antagonist AP5 (10 μM). In addition, our results indicate that NMDA receptor activation is not required for normal nerve cell growth, since the growth of neurons in the presence of AP5 was not significantly different from control, but is involved in the potentiation of normal nerve cell growth. Results of these investigations indicate that the neurosteroid, PS, can act as a significant neurotrophic factor for hippocampal neurons.

Supported by grants from the Norris Foundation and the Schuster Foundation to R.D.B.

703.11

GANGLIOSIDES REGULATE CYTOSKELETAL STRUCTURE AND PROTEINS IN NEURO-2a NEUROBLASTOMA. L-J. Wang, R. Colella & F.J. Roisen.* Anatomical Sciences & Neurobiol., Univ. of Louisville School of Med., Louisville, KY 40292.

It is well established that gangliosides enhance neuritogenesis of primary neuronal tissues and several cell lines, but the exact mechanism remains unknown. Our previous studies demonstrate that ganglioside exposure simultaneously increases the microtubule network and neuritogenesis of Neuro-2a cells. A redistribution of MAP2 immunoreactivity from the perikaryon to the distal processes occurs following 24 hr GM1 treatment. In contrast, MAP5 and tau immunolocalization remains unchanged. Immunoelectron microscopy demonstrates that MAP2 is more closely associated with actin-rich subcortical cytoplasm than with microtubules after GM1 exposure (more MAP2 label per unit area was found in spine-like projections and in neurites). To determine if GM1 alters neuronal development by regulating the induction of synthesis of the cytoskeletal proteins or by targeting specific cytoskeletal proteins to distinct regions in the neuron, protein and RNA analysis is in progress. Western blot analysis reveals increased levels of acetylated tubulin but not MAP2 following GM1 exposure. Actin mRNA increases in a GM1 dose dependent fashion. Studies in progress examine the time dependent effects of GM1 exposure on actin levels and topography via immunolocalization. Our data suggests that gangliosides may be involved in the determination of dendritic or axonal fate by selectively changing the distribution of MAP2, stabilizing microtubule networks and increasing actin synthesis. It is possible that gangliosides enhance neurite formation by altering the balance of microtubules and microfilaments through MAP2-dependent mechanisms. Supported by Alliant Community Trust Fund, Louisville, KY.

703.12

ANALYSIS OF RECEPTOR TYPE TYROSINE KINASE AND ITS LIGAND DURING EARLY MOTONEURON DEVELOPMENT. K. Ohta*, H. Iwamasa, M. Nakamura, N. Iino, and H. Tanaka. Divi. of Dev. Neurobiol., Dept. of Neurosci. and Immunol., Kumamoto Univ. Grad. Sch. of Med. Sci., Kumamoto 862, Japan.

Receptor tyrosine kinases (RTKs) play important roles in cellular survival, proliferation, and differentiation. We have isolated 19 RTKs by RT-PCR method from E5 chick motoneurons. In situ hybridization analysis revealed that chick Sek, a member of eph family, was expressed from E5 on motoneurons at the brachial and lumbar segments of the spinal cord which innervate limb muscles, and disappeared after the naturally occurring cell death period (E6-E11). Immunohistochemistry using anti-chick Sek monoclonal antibody showed the localization of chick Sek protein at cell bodies and axonal fibers of motoneurons. We also isolated a chick homolog of ELF-1, which was identified as a ligand for both Mek4 and mouse Sek. Chick ELF-1 was 72% identical to mouse ELF-1. While the expression of chick Sek was highly regulated in time and space, chick ELF-1 was widespread. The unique expression of chick Sek suggests the involvement of it in the cell-cell interactions for specific subpopulations of developing motoneurons.

703.13

p55^{PIK},A NEW PI3K REGULATORY SUBUNIT ENGAGES IGF-I SIGNALING DURING CENTRAL NERVOUS SYSTEM DEVELOPMET. S.Pons., J.Torres-Aleman*., M.F.White.
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Phosphatidylinositol (PI-3) kinase is implicated in the regulation of diverse cellular processes, including neurite outgrowth in PC12 pheochromocytoma cells. PI-3 kinase is composed of a 110-kDa catalytic subunit and an 85-kDa regulatory subunit. Here we describe p55^{PIK}, a new regulatory subunit that was isolated by screening expression libraries with tyrosine phosphorylated IRS-1. The p55^{PIK} is composed of a unique 30-residue NH_2-terminus followed by a proline-rich motif and two SH2 domains which contain homology to those of p85.

In contrast to the ubiquitous expression of the previously described p85, expression of p55^{PIK} is mainly in the central nervous system (CNS). Maximum levels of expression are found between 13.5-15.5dpc, decreasing in subsequent developmental stages with only minimal levels detected in the adult brain. High levels of p55^{PIK} expression are temporally and spatially coincident with neuronal differentiation in CNS development.

The p55^{PIK} forms a stable complex with p110, and associates with IRS-1 during IGF-I stimulation in both primary neuronal cultures and in P19 cells differentiated to neuronal fate. The unique features of p55^{PIK} suggests that it may be important in IGF-I receptor signaling during neuronal differentiation.

703.14

EXPRESSION OF INTERLEUKIN-3 RECEPTOR ALPHA AND BETA IN MURINE CENTRAL NERVOUS SYSTEM NEURONS. Y. Konishi*[1], T. Kunishita [1], D.-H. Chui [1], S. Yonehara [2], K. Takahashi [1] and T. Tabira [1]. [1] National Institute of Neuroscience, NCNP, Tokyo 187, Japan. [2] Institute for Virus Research, Kyoto University, Kyoto 606, Japan.

We have previously reported that interleukin-3 (IL-3) is one of the candidates for cholinergic neurotrophic factors (Neuron 4:429-436, 1990). Since that report, it has been questioned whether IL-3 acts directly on cholinergic neurons or indirectly on them via other populations of cells in the central nervous system (CNS). We now demonstrate the expression of IL-3 receptor (IL-3R) alpha (SUT-1) and beta (AIC-2A and -2B) in the CNS neurons by means of reverse transcription and polymerase chain reaction (RT-PCR) and immunohistochemistry, although the partial data on IL-3R beta were already reported (Neurosci. Res. 19:S137, 1994; Int. J. Dev. Neurosci. 1995, in press). RT-PCR followed by Southern blot analyses revealed that the mRNAs for IL-3R alpha and beta are found in an embryonic cholinergic septal cell line SN6 and primary cultured neurons derived from the septal regions of embryonic mice. In contrast, a postnatal cholinergic septal cell line SN49 expresses only IL-3R alpha mRNA, and a postnatal non-cholinergic septal cell line SN52 expresses none of the mRNA for IL-3R alpha and beta (SN6, SN49, SN52 are kind gifts from Dr. B. H. Wainer, Albert Einstein College of Medicine, Bronx, NY). Actually IL-3 elevates choline acetyltransferase (ChAT) activity in SN6 but not in SN49, 52. These results indicate that IL-3 is utilized by cholinergic neurons via a set of IL-3R alpha and beta expressed in the cholinergic neurons. The results were supported by immunohistochemical study showing that IL-3R alpha and beta were found in cholinergic neurons of the basal forebrain. (Supported partially by the Funds from the Science and Technology Agency, the Health Sciences Foundation, and the Naito Foundation in Japan.)

703.15

CHARACTERIZATION OF A NOVEL AUTOCRINE INTERACTION OF HEPATOCYTE GROWTH FACTOR/SCATTER FACTOR AND ITS RECEPTOR, MET IN SUPERIOR CERVICAL GANGLIA PRIMARY CULTURE. X.-M. Yang[1], D. Belliveau[1], J. Kohn[1], M. Park[2] and F.D. Miller[1]*. 1: Centre for Neuronal Survival, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada. H3A 2B4. 2: Molecular Oncology Group, Royal Victoria Hospital. Department of Oncology, Medicine and Biochemistry, McGill University.

Hepatocyte growth factor (HGF)/scatter factor (SF) is a multifunctional cytokine that stimulates mitogenesis, motogenesis, morphogenesis of a broad spectrum of epithelial and endothelial cells. The HGF/SF cellular responses are mediated by the Met receptor tyrosine kinase. To investigate the possible biological function(s) of the HGF/SF and its receptor in the nervous system, we chose to analyse the expression of the HGF/SF and its receptor in primary culture of sympathetic neurons from the superior cervical ganglia (SCG). RT-PCR demonstrated that sympathetic neurons express HGF/SF mRNA. Moreover, condition media of sympathetic neuron cultures contained bioactive HGF/SF as indicated using a scatter assay with MDCK cells. Sympathetic neurons also synthesize met receptor mRNA, and immunofluoresence studies indicate that the Met receptor is localized to both cell bodies and neuritic processes. Thus, sympathetic neurons synthesize both HGF/SF and its receptor, Met, suggesting the possibility of a novel autocrine loop. The possible biological function of such an autocrine loop is under investigation in sympathetic neurons in culture and in vivo.

703.16

EXPRESSION OF THE INTRACELLULAR DOMAIN OF THE P75 NEUROTROPHIN RECEPTOR IN TRANSGENIC MICE PERTURBS NEURONAL DEVELOPMENT. Freda D. Miller, Audrey Speelman, Christine Zeindler, Shernaz Bamji, Daniel Belliveau, Christian Lachance, Andrew Gloster*, and Philip A. Barker. Center for Neuronal Survival, MNI, McGill University, Montreal, Canada H3A 2B4.

Intracellular domains of receptors can act as autonomous signalling units. As a means of assessing p75 neurotrophin receptor signalling functions in vivo, we have produced transgenic mice which overexpress the intracellular domain of p75 (ICD) within neurons. Specifically, we have utilized the Tα1 α-tubulin promoter, which is expressed at high levels in early developing neurons, and shows reduced expression after neurons mature. Analysis of four lines of transgenic mice generated using the Tα1:ICD construct revealed that at least two of these lines express the transgene at readily detectable levels. Animals from both lines display ptosis and are smaller and noticeably less coordinated than their control littermates. In both of these lines, the sensory and sympathetic ganglia are significantly smaller than in control littermates. Cell counts revealed that transgenic sympathetic ganglia contain approximately 30% fewer neurons than control littermates. Analysis of sensory neurons of the DRG indicated 60% cell loss, preferentially of small neurons. To determine whether the CNS of these animals is also affected, Tα1:ICD mice were crossed with a previously-characterized line of transgenic mice that expressed ß-galactosidase from the same promoter (line K6, Tα1:nlacZ animals, as reported in Gloster et al., J. Neurosci., 14, 7319, 1994). Histochemical staining with Xgal revealed that there are significant perturbations in neuronal distribution throughout the brains of the Tα1:ICD mice. For example, the laminar distribution of neurons within the cortex is disturbed and occasional clusters of blue nuclei are observed within the white matter. The cellular basis for these perturbations in neuronal development is currently under investigation.

703.17

A Cytoplasmic Homology Between p75^{LNTR}, TNF-RI and the Fas Antigen May Define Analogous Functional Domains. P.A. Barker*, C. Zeindler, D. Kryl, C. Lachance. Center for Neuronal Survival. Montreal Neurological Institute. McGill University, Montreal, Quebec. H3A 2B4

p75^{LNTR} was the first identified member of a superfamily of related receptors which includes CD27, CD30, CD40, 4-1BB, OX40, the fas antigen and the two tumor necrosis factor receptors (TNF-R1 and TNF-R2). The shared homology that defines this group resides in conserved protein modules, termed Cys repeats, which are present extracellularly. p75^{LNTR} has recently been shown to signal autonomously by stimulating production of the lipid second messenger, ceramide. In this respect, p75 can be grouped with two other members of the TNF receptor superfamily, TNF-R1 and fas.

Consistent with this conserved functional homology, we have found that the intracellular domain of the p75^{LNTR} bears considerable sequence homology to the previously described "death domain" of the TNF-R1 and fas receptors. Use of the MACAW program (NCBI) reveals three regions of statistically significant homology between these three receptors. All of these small domains are located within the "death domain" region previously defined for TNF-R1 and fas. Parsimony analysis indicates that p75^{LNTR} and TNF-R1 are as closely related within this region as are TNF-R1 and fas.

Motifs allowing receptor oligomerization are present within the TNF-R1 and fas intracellular domains. To determine if the intracellular domain, or portions thereof, are capable of self-association, interactions have been assayed in vitro using bacterial fusion proteins and in vivo using the yeast two hybrid system. The identification of this conserved intracellular domain within p75^{LNTR} should aid future investigations of the signaling capabilities of each of these related receptors.

703.18

LAR RECEPTOR-DEFICIENT TRANSGENIC MICE: DECREASED LAR EXPRESSION AND ABERRANT DENTATE GYRUS INNERVATION. T. Yang[1], T.T. Yeo[2], L.L. Butcher[2], J.S. Zhang[1] and F.M. Longo*[1]. [1]Dept. of Neurology, UCSF/VAMC, San Francisco, CA 94121 and [2]Dept. of Psychology, UCLA, Los Angeles, CA 90095.

Developmentally-regulated expression of the Leukocyte Common Antigen-Related (LAR) tyrosine phosphatase receptor and neuron-preferential alternative splicing suggests that LAR regulates mammalian neural development (Longo et al. SNS, 1991; Longo et al, JBC, 1993; Zhang and Longo, JCB, 1995). LAR transgenic mice (DBA2/129Ola hybrids) were created by W. Skarnes using a gene trap that prevents synthesis of normal LAR transcripts (Centre Genome Research, Edinburgh) and bred in our laboratory. Northern analysis of brain RNA from LAR homozygous (-/-) transgenic mice showed trace or absent ~8kb LAR transcript as compared to the wild type (+/+, DBA2). Dentate gyrus innervation from basal forebrain was examined by immunohistochemistry using antibody against the p75 NGF receptor which co-localizes with cholinergic markers. In -/- mice we observed: i) reduction of p75 immunoreactivity in the infragranular band; ii) disrupted laminar pattern of fibers entering the granule cell layer; iii) fiber thickening with aberrant beading in granular and polymorphic layers, raising the possibility of a neurodegenerative process. Heterozygous intercrosses will determine if strain difference account for any of these abnormalities. These observations support our hypothesis that LAR-type tyrosine phosphatases regulate neurite outgrowth and/or synapse formation. Supported by an AFAR Beeson Award (FL), NIA R01 (FL), VA (FL), French Fdn (TTY) and Retirement Research Foundation (LB).

703.19

LAR TYROSINE PHOSPHATASE RECEPTOR: RNA AND PROTEIN EXPRESSION OF LAR ALTERNATIVELY SPLICED ELEMENT-C (LASE-C). J.S. Zhang*, J. Honkaniemi, T. Yang and F.M. Longo. Dept. of Neurology, UCSF/VAMC, San Francisco, CA 94121.

The Leukocyte Common Antigen-Related (LAR) tyrosine phosphatase receptor contains Ig and fibronectin type III domains resembling those in cell adhesion molecules mediating neurite outgrowth. Developmentally regulated expression of rat LAR in neurons suggests a role in neural development (Longo et al SNS Abst, 1991; Longo et al JBC, 1993). LASE-c is a 27 bp alternatively spliced exon in the fifth fibronectin type III LAR domain. Its splicing is regulated by development, NGF and denervation and preferentially occurs in neural tissue (Zhang and Longo JCB 1995). *In-situ* hybridization with a LASE-c probe which identifies the ~7 kb LAR transcript on Northern blots showed diffuse neuronal expression at birth. In adults, however, high expression was largely restricted to subsets of neurons in the hippocampus and vestibular, reticular thalamic, oculomotor and deep cerebellar nuclei. Northern analysis showed markedly reduced LASE-c expression in cortex, hippocampus and cerebellum during postnatal development. On Western blots, affinity-purified antiserum raised against LASE-c peptide identified the predicted 150kD LAR band and preferentially stained neurons in dorsal root ganglia. LASE-c antibody also stained sciatic nerve neurites and neurites and growth cones of cultured neurons. Expression of LASE-c in specific subsets of neurons and presence of LAR protein with the LASE-c domain in neurites and growth cones supports our hypothesis that highly-regulated alternative splicing of LAR-type tyrosine phosphatases regulates neurite outgrowth and/or synapse formation. Supported by American Paralysis Association (FL) and NIA R01 (FL).

703.20

PHOSPHORYLATION OF GP130 IN RESPONSE TO LEUKEMIA INHIBITORY FACTOR OR MITOGEN STIMULATION OF 3T3-L1 CELLS. W.P. Schiemann*, and N.M. Nathanson. Department of Pharmacology, Univ. of Washington, Seattle, WA 98195.

We have demonstrated recently that the human LIF receptor (LIFR) is phosphorylated at Ser1044 by mitogen-activated protein kinase (MAPK) *in vitro*, and that heterologous receptor-mediated attenuation of LIFR-stimulated reporter gene expression occurs through effects at Ser1044 *in vivo*. We have constructed bacterially expressed fusion proteins containing the entire or various cytoplasmic regions of human gp130, the β-subunit of activated LIFRs, and measured their ability to serve as substrates in protein kinase reactions. We found that gp130 fusion proteins, like those for the LIFR, were phosphorylated in extracts of LIF- or growth factor-stimulated 3T3-L1 cells predominantly on Ser residues C-terminal to box 3 in a dose- and time-dependent manner that mirrored the activation of MAPK. The gp130 protein kinase(s), however, is clearly distinct from activated MAPK because: (i) gp130 was an extremely poor substrate for active recombinant MAPK *in vitro*; (ii) gp130 phosphotransferase activity was readily separated from the MAPK isozymes ERK1 and ERK2 following Mono Q or Superose 12 chromatography; and (iii) Ca^{++}/phospholipid were required to reconstitute phosphorylation of gp130 fusion proteins in fractionated extracts. Additionally, we find that pretreatment of 3T3-L1 cells with growth-factors coupled to activation of the MAP kinase cascade blocked ~75% of LIF-stimulated Tyr phosphorylation of LIFR and gp130. Preincubation of 3T3-L1 cells with phorbol ester or thapsigargin, agents which activate the MAP kinase cascade in a cell surface receptor-independent manner, also inhibited LIF-stimulated Tyr phosphorylation in LIFR immunoprecipitates. Our results suggest that the gp130 polypeptide is phosphorylated by a protein kinase whose activation is tightly coupled to the stimulation of MAPK, and that stimulation of Ser/Thr phosphorylation of LIFR and gp130 following activation of MAP kinase cascade-coupled receptor systems may serve as a major regulatory switch governing LIFR signaling *in vivo*.

703.21

INTERLEUKIN-1β RECEPTOR MEDIATED NGF SECRETION FROM RAT CORTICAL ASTROCYTES IN PRIMARY CULTURES. M. Čarman-Kržan*, V. Pahor, D. M. Seliškar. Dept. Pharmacology, Medical Faculty, Korytkova 2, Ljubljana, Slovenia.

Interleukin-1β (IL-1β) is one of the most potent stimulators of NGF secretion from rat neonatal cortical astrocytes in primary cultures . Our previous studies showed that IL-1β acts at the level of NGF gene transcription and suggested glial IL-1 receptor mediated mechanism (Čarman-Kržan M and Wise BC. J Neurosci Res 34, 1993, 225-232). Using iodinated recombinant human IL-1β in the binding studies we identified IL-1β receptors on the astrocyte cell population (B_{max}=2.6 ±0.6 fmol/mg protein, K_D=2.58 pM). Further characterization of IL-1β receptors on astrocytes with inhibition binding studies showed that rh IL-1β is the most potent inhibitor (K_i=0.74 nM) followed by rh IL-1Ra (K_i=2 nM), rh IL-1α (K_i=7.9 nM) whereas rh IL-2 has no inhibitory action on [^{125}I] rh IL-1β labeled IL-1β receptor. Their inhibitory action on IL-1β receptor is in good correlation with their ability to induce or inhibit NGF secretion from these cells: rh IL-1β (10 U/mL) is the most potent activator of NGF secretion from astroglial cells (3 fold over basal level) followed by rh IL-1α. rhIL-1Ra and IL-2 alone failed to stimulate NGF release. Our further studies showed that rh IL-1β stimulated NGF release is inhibited by the presence of rh IL-1Ra in a dose dependent manner, confirming that IL-1 induced NGF secretion from astrocytes is IL-1β receptor mediated process.

NEURONAL DEATH VIII

704.1

Role of Interleukin 1β converting enzyme in neuronal cell death

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Interleukin-1β converting enzyme (ICE), the mammalian homolog of ced-3, is formerly known as the enzyme responsible for the production of IL-1β. Recently our studies demonstrated that ICE activity participates in neuronal cell death (CD) and inhibiton by crmA can delay this process (Gagliardini et al., Feb 11 Science 263, 1994). In order to better understand the role of ICE in programmed CD, we demonstrated an induction of ICE during apoptosis in dorsal root ganglion (DRG) neurons. A basic level of ICE was detected in neurons and the expression of ICE was upregulated in dying neurons with condensed nuclei. The requirement of ICE/ced-3 family genes in neuronal programmed CD is supported by the ability of the precursor of IL-1β (pIL-1β) or mutant ICE with a point mutation in ICE active site (C to G), that elimimate pIL-1β processing activity as well as autoprocessing, to prolongue neuronal survival. Microinjection of the human pIL-1β gene as well as the mutant form of mouse ICE into chicken DRG neurons was found to prevent CD induced by deprivation of nerve growth factor. Neurons were able to survive for 6 days often with neurites and healthy cell body.

These evidences suggest that ICE is playing a major role in neuronal CD. Further experiments have to be done to understand whether additional members of the ICE/ced-3 family are also involved in controlling neuronal CD.

704.2

GENE EXPRESSION IN NEURONS DURING PROGRAMMED CELL DEATH. R. S. Freeman* and R. J. Crowder. Department of Pharmacology, University of Rochester, School of Medicine, Rochester, NY 14642.

The expression of certain genes is upregulated during programmed cell death (PCD) in sympathetic neurons deprived of nerve growth factor (NGF) (Freeman et al., *Neuron* 12:343-355, 1994; Estus et al., *J. Cell. Biol.* 127:1717-1727, 1994). These genes are induced despite an overall decrease in RNA and protein synthesis soon after NGF withdrawal. To identify other genes that are preferentially expressed in dying neurons, we have utilized the technique called differential display as a means of comparing the mRNAs present in NGF-maintained versus NGF-deprived neurons. With this technique, we have identified a new gene, termed DD1-X, that is upregulated after NGF withdrawal. DD1-X mRNA levels begin to accumulate within 5 hours after NGF withdrawal and peak at about 10 hours. To test the involvement of DD1-X (and other genes, such as cyclin D1, c-jun, c-fos, and c-myb) in the cell death process, we have examined its expression in neuronal death paradigms that are independent of NGF withdrawal. For example, cytosine arabinoside (AraC) kills postmitotic neurons in a manner that resembles NGF deprivation except that the onset of death is delayed by many hours. DD1-X, cyclin D1, c-myb, c-fos, and c-jun are all upregulated during AraC induced neuronal death. Furthermore, the induction of these genes is delayed relative to their induction during NGF deprivation-induced PCD, consistent with their potential involvement in the cell death process. To test DD1-X and cyclin D1 for a direct role in the mechanism for PCD, we are attempting to inhibit their expression in NGF-deprived neurons using antisense approaches.

704.3

PC12 CELLS, TRANSFECTED WITH CALBINDIN-D28k cDNA, ARE PROTECTED FROM DEGENERATION CAUSED BY SERUM WITHDRAWAL. A. McMahon, E. Lephart*, C.L. Liang, and D.C. German. Dept Psychiat, UT Southwestern Med Sch, Dallas, TX 75235-9070.

Calbindin-D28k (CALB) is a calcium-binding protein that is found in many neurons, and it has been proposed to play a neuroprotective role. We sought to identify a tissue culture system in which to directly study the role of CALB. PC12 cells normally express low levels of CALB, as detected by Western analysis. After treatment with NGF, however, there was a maximum 6 fold increase above control levels by 4 days. Greater levels of CALB expression were obtained by stably transfecting PC12 cells with a rat cerebellar CALB cDNA. Two pools of cells were generated, calb-70 and calb-58, containing >100 fold increased CALB protein. These cells, and control cells, were incubated for 24 hours in serum depleted media. When cell integrity was assayed by measuring release of lactate dehydrogenase (LDH), control cells gave a 3 fold increase, calb-70 cells a 2 fold increase, and calb-58 cells a 1.5 fold increase. These data suggest that CALB: (1) can interfere with apoptosis (since serum depletion kills cells by activating apoptotic mechanisms); and (2) may be important in mediating the neuroprotectant effects of NGF.

704.4

Nitric Oxide Generating Compounds Prevent the Death of PC12 Cells and Primary Sympathetic Neurons Following Withdrawal of Trophic Support. S.E. Farinelli, M.P. Joyce* and L.A. Greene. *Dept. of Pathology and Ctr. for Neurobiology and Behavior, Columbia University, New York, NY 10032,.*

We have used both the PC12 cell line and primary cultures of rat sympathetic neurons as model systems to examine the mechanism by which neuronal cells undergo apoptotic death when deprived of growth factors. Previously we have provided evidence for a possible link between uncoordinated cell cycle progression and apoptotic death in these systems including data which suggests that certain cell cycle blockers protect cells from death. Since it has been reported that nitric oxide (NO) generating compounds inhibit proliferation of a number of cell types, we proceeded to test these compounds for their ability to protect PC12 cells and sympathetic neurons following removal trophic support. Two such agents S-nitroso-N-acetylpenicillamine (SNAP) and diethylenetriamine nitric oxide adduct (DETA-NO) provide complete protection for at least two days following removal of serum from naive PC12 cells while only 15% of the untreated cells remained alive. These compounds also provided complete protection for two days to neuronally-differentiated PC12 cells following withdrawal of NGF in serum-free medium while only 50% of the untreated cells survived. Treatment of primary sympathetic neurons with DETA-NO and SNAP following withdrawal of NGF also resulted in significantly enhanced survival relative to controls. A well understood target of NO action is guanylate cyclase which is activated by nitrosylation of its heme prosthetic group. LY-83,583, an inhibitor of guanylate cyclase, blocks the protective effects of both SNAP and DETA-NO on serum-deprived PC12 cells without preventing NGF-induced survival, suggesting that the protective effects of NO may be mediated by elevated intracellular cGMP. (Supported by grants from the NINDS).

704.5

INDUCTION OF *p53*-DEPENDENT NEURONAL APOPTOSIS BY THE ANTIMITOTIC CYTOSINE ARABINOSIDE, WHOSE TOXICITY CLOSELY RESEMBLES DEATH BY TROPHIC FACTOR DEPRIVATION. T.L. Deckwerth* and E.M. Johnson, Jr. Washington University School of Medicine, Department of Molecular Biology and Pharmacology, St. Louis, Missouri 63110, USA.

Exposure of nerve growth factor (NGF)-maintained neonatal rat sympathetic neurons to the antimitotic agent cytosine arabinoside (araC) resembles death by NGF deprivation based on pharmacological criteria (Martin *et al., J. Neurosci.* 10: 184-193, 1990). Here we show that the similarities between death by NGF deprivation, which occurs by apoptosis (Deckwerth and Johnson, *J. Cell Biol.* 123: 1207-1222, 1993), and by exposure to araC extend to several biochemical and morphological parameters indicative of apoptotic neuronal death. Similar to NGF-deprivation, araC-induced apoptosis was accelerated in murine sympathetic neurons deficient in the death suppressor *bcl2*. To determine whether araC might induce apoptosis by inflicting DNA damage, we examined whether the gene dose of the tumor suppressor *p53*, which triggers apoptosis upon excessive DNA damage, would affect the kinetics of death after treatment with araC. In the absence of *p53*, neuronal death by exposure to araC was delayed significantly, consistent with a potential role for DNA damage in contributing to araC-induced neuronal apoptosis. Supported by the AT Children's Project and NIH grant NS24769.

704.6

APOPTOSIS OF SERUM DEPRIVED PC12 CELLS AND NGF RESCUE: EFFECTS ON AP-1 DNA BINDING ACTIVITY. L. Tong*, K. Werrbach-Perez and J.R. Perez-Polo. Dept. of HBC&G, UT Med. Branch, Galveston, TX 77555-0652

We have previously reported that transcription factor AP-1 DNA binding activity transiently increased and then decreased during apoptotic cell death induced by serum deprivation. NGF, which rescues PC12 cells from apoptosis, upregulated AP-1 binding activity persistently. Our hypothesis is that impaired AP-1 DNA binding activity after serum deprivation may contribute to apoptosis in PC12 cells and that NGF may rescue cells via maintenance of AP-1 DNA binding activity. K252a, an inhibitor of the Trk receptor, abolished both NGF rescue of PC12 cells and increases in AP-1 DNA binding activity. Enhanced AP-1 activity in the presence of combined NGF and protein synthesis inhibitor cycloheximide treatment indicated that there is persistent AP-1 activation due to posttranslational modifications of various *fos* and *jun* proteins. Increased AP-1 DNA binding activity also was persistent during Insulin and cAMP-mediated survival of PC12 cells after serum deprivation. In addition, there was a decline in AP-1 activity in PC12 cells after NGF deprivation. Furthermore, retinoic acid, known to have a repressive function on AP-1 activity, abolished NGF-mediated rescue and increased the sensitivity of PC12 cells to serum deprivation. Therefore, serum deprivation-induced cell death and NGF rescue correlate with AP-1 DNA binding activity. Supported in Part by NINDS-NS18708. This is publication 35A from USPHS grant P01 AG10514 awarded by NIA.

704.7

REDUCING AGENTS PREVENT APOPTOTIC DEATH OF TROPHIC-FACTOR-DEPRIVED PC12 CELLS AND SYMPATHETIC NEURONS. C.Y.J.Yan*,G.Ferrari,and L.A.Greene. Dept. of Pathology, Columbia University, Coll. of P & S, New York, NY 10032.

We have used serum-deprived PC12 cells and NGF-deprived sympathetic neuron cultures as models to study apoptosis elicited by trophic factor withdrawal. In our paradigm, N-acetyl-cysteine (NAC) maintains long-term survival of PC12 cells and sympathetic neurons and also inhibits PC12 cell proliferation. NAC has been reported to be an anti-oxidant that increases intracellular glutathione (GSH) levels. However, several other anti-oxidants do not promote survival or block proliferation, suggesting that NAC's actions cannot be explained by its anti-oxidant properties. The NAC-mediated increase in intracellular GSH also seemed unnecessary since depleting intracellular GSH with buthionine sulfoximine did not affect NAC's protective or anti-proliferative effects. An alternative explanation is that NAC acts as a reducing agent. When we tested other compounds with reducing properties, a number retarded death. Among the most effective was 2,3 dimercaptopropanol (British anti-Lewisite). This compound also blocked PC12 cell proliferation. These findings suggest that NAC prevents death by means of its reducing activity and that other reducing agents may also inhibit the apoptotic pathway. Finally, these data indicate that like NAC, other reducing agents block DNA synthesis and this activity may account for their capacity to rescue neuronal cells from apoptotic death. Supported by the NINDS and ALS Foundation.

704.8

CHANGE OF INTRACELLULAR cAMP LEVELS IN SYMPATHETIC NEURONS UNDERGOING PROGRAMMED CELL DEATH J.Y.Chang* and V.V.Korolev Dept. Anatomy, Univ. Arkansas for Medical Sciences, Little Rock, AR 72205

The survival and proper functioning of sympathetic neurons is dependent on nerve growth factor (NGF). When immature sympathetic neurons are deprived of NGF, they undergo an "active" dying process usually termed "programmed cell death" (PCD), or "apoptosis". Cyclic AMP or agents capable of increasing intracellular cAMP can prevent this sympathetic neuronal PCD caused by trophic factor deprivation. This trophic dependence is age-related such that the cells become less dependent on NGF as they mature. Sympathetic neuronal cultures derived from rat superior cervical ganglia were used to study the relationship between intracellular cAMP levels and neuronal cell death. With a cAMP radioimmunoassay, our results indicate that as a neuron matures, the relative amount of cAMP per cell increases concurrently. There is a 2-fold increase of cAMP levels per cell when the cAMP levels from a 1-week- and 2-week-old culture were compared. Removal of NGF in 1-week-old cultures, which triggers the process of PCD, results in a decrease of intracellular cAMP levels. NGF deprivation for 24 and 48 hours leads to a 50% and 75% decrease of cAMP levels. In contrast, when these cells mature in culture and become relatively NGF independent (i.e., 2-week-old culture), NGF withdrawal does not lead to a drop of cAMP levels. PACAP (pituitary adenylate cyclase-activating peptide) , a naturally occurring bioactive peptide, can increase cAMP levels in these sympathetic neurons, and prevent neuronal cell death resulting from NGF deprivation. (Supported by NIH NS32253)

704.9

METABOLIC AND GENETIC ANALYSES OF APOPTOSIS IN POTASSIUM/SERUM-DEPRIVED CEREBELLAR GRANULE CELLS

T.M. Miller* and E.M. Johnson, Jr. Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

Cerebellar granule cells may be maintained in serum-containing medium by elevating (25 mM) extracellular potassium levels. Removal of both potassium and serum after seven days in culture triggers a characteristic apoptotic death (D'Mello et al., 1993, P.N.A.S. **90,** 10989–10993) We have found that removal of the serum has an important function in this model of apoptosis. After 96 hours of potassium deprivation in the presence of serum, 40–45% of the cells are still alive whereas more than 95% of the potassium/serum-deprived cells have died by this time. Thus, while the nearly complete cell death of the potassium/serum-deprivation system provides a convenient model, this system is complicated by removing two sources of trophic support at the same time.

Metabolic and genetic parameters are being analyzed in the potassium/serum-deprivation model. Protein synthesis decreases precipitously after potassium/serum-deprivation to less than 15% of control (25mM K^+ plus serum) at 12 hours, a time when approximately 50% of the neurons are committed to die. Cells maintained in 25mM K^+ without serum showed a slow decline in protein synthesis to approximately 50% at 24 hours. *C-jun*, a gene required for cell death after trophic factor deprivation is also induced several fold in potassium/serum-deprived cerebellar granule cells. Similarities and differences in the pattern of gene expression in dying sympathetic and granule neurons will be presented. Supported by NIH grants NS 24679 and AG 12947 and by the Washington University ADRC (P50 AG 5681).

704.10

DENSITY-DEPENDENT PROTECTION OF RAT CORTICAL NEURONS FROM APOPTOSIS IN SERUM-FREE PRIMARY CULTURES. N. Fukushima*, H. Ueda, Y. Sasaki and Y. Misu, Dept. of Pharmacol., Yokohama City Univ. Sch. of Med., Yokohama 236,

Neurons are known to require exogenous signals, such as serum, trophic factors or glial cells, for survival. We have reported that the neuronal cell death, which occurs in the serum-free primary cultures of rat cortex without any supplements, was inhibited in a cell density-dependent manner. In the present work we further characterized the density-dependent inhibition of the cell death. When the rat cortical cells were seeded at the low density (1 x 10^5 cells/cm^2), the number of neurons (neurofilament-positive cells) markedly decreased within 6 hours after seeding. Since most of neurons were not stained with propidium iodide, they were found to be viable. The DNA ladders, biochemical feature of apoptosis, were detected 3 hours after seeding. However, the rapid cell death was inhibited in a density-dependent manner in the ranges of 1 to 5 x 10^5 cells/cm^2. In addition, DNA laddering was inhibited in the high density (5 x 10^5 cells/cm^2) cultures. Both glial and proliferative cells were negligible in all cultures. These findings indicate that cortical neurons are protected from apoptosis in a density-dependent manner under the serum-free condition.

704.11

SERUM SUBSTITUTES FOR ENDOGENOUS FACTORS INVOLVED IN CELL DENSITY-DEPENDENT SURVIVAL OF RAT CORTICAL NEURONS. Y. Sasaki[1,2]*, H. Ueda[1], N. Fukushima[1], T. Okada[2], Y. Misu[1]. [1]Department of Pharmacology, Yokohama City University School of Medicine, Yokohama 236, Japan, [2]International Research Laboratories, Ciba-Geigy Japan, Takarazuka 665, Japan.

We have reported that survival of neurons was cell density-dependent in the primary culture of rat cortex under serum-free condition in this meeting last year. To examine mechanism of neuronal cell death at low density culture (1 x 10^5 cells/cm^2), we developed a new colorimetric assay for survival, using Alamar Blue. Alamar Blue is a redox indicator which yields a colorimetric change in response to chemical reductants derived from living cells. At low density culture, the rate of neuronal survival decreased to 20% of initial neurons at 15 hr. In this process of neuronal cell death, genomic DNA laddering was observed, indicating that neurons are dying in a manner of apoptosis. On the other hand, more than 80% of initial neurons survived at higher density culture (5 x 10^5 cells/cm^2) at 15 hr. In the presence of 10% serum, however, survival of neurons increased at lower density and unchanged at high density. As a result, there was no more density-dependent survival. Survival of neurons at low density was increased by addition of 10% serum only for initial 1 hr and deprivation of serum subsequently, while the survival was not enhanced by the addition of serum at 1 hr after seeding. These findings suggest that serum substitutes for endogenous factors involved in cell density-dependent survival of neurons.

704.12

REGULATION OF APOPTOSIS IN THE NEURONAL LINEAGE OF MOUSE OLFACTORY EPITHELIUM. J. D. Holcomb*, J.S. Mumm and A.L. Calof. Dept. of Biological Sciences, Univ. of Iowa, Iowa City, IA 52242.

When olfactory epithelium (OE) is deprived of interaction with its target tissue, the olfactory bulb (OB), cells in the OE die. We have characterized the timecourse and extent of this induced cell death in vivo, following unilateral ablation of the olfactory bulb (OBX) in adult mice, and in vitro, in cultures of dissociated neuronal cells isolated from E16.5-17.5 OE. Cells undergoing apoptosis were detected using end-labeling of fragmented DNA (TUNEL staining); ^{3}H-thymidine incorporation was used to label neuronal precursors, and cell type-specific antibodies were used to distinguish different OE cell types. In vivo, cells at all stages in the neuronal lineage of the OE -- neuronal precursors, immature olfactory receptor neurons (ORNs), and mature ORNs -- were found to undergo apoptotic death. Apoptosis occurs at low levels in all neuronal cell types in unoperated OE and increases dramatically in all neuronal cell types at short times (24 hr) following OBX; at long times (8 wks) post-OBX, however, apoptosis is increased exclusively in mature ORNs. In vitro, both neuronal precursors and ORNs undergo apoptosis when cultured in the absence of OB or added trophic factors; this can be inhibited by aurintricarboxylic acid (ATA), a known inhibitor of apoptosis in many types of cells. Both ATA and a membrane-permeant analog of cyclic AMP (CPT-cAMP) promote survival of a substantial fraction of cultured ORNs at 72 hrs in vitro. Survival of ORNs is also promoted by certain neurotrophins (BDNF, NT-3, and NT-5, but not NGF), although none promote survival to as great an extent as ATA or CPT-cAMP. Consistent with this, expression of trkB was observed in a subset of ORNs in neonatal OE. These results suggest that apoptosis is a mechanism for regulating cell number at all developmental stages in the ORN lineage, and implicate one class of polypeptide growth factor -- the neurotrophins -- in this process. We are currently testing neurotrophins in vivo, to determine whether they can rescue ORNs from apoptotic death induced by OBX.

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704.13

CEP1347 PREVENTS CELL DEATH INDUCED BY NGF-DEPRIVATION IN DORSAL ROOT GANGLION NEURONS AND PC12 CELLS IN CULTURE M.A. Glicksman[1]*, M.E. Forbes[1], T. O'Kane[1], C. Murakata[2], N.T. Neff[1], D. Bozyczko-Coyne[1]. [1]Cephalon, Inc, West Chester, PA 19380; [2]Kyowa-Hakko Kogyo, Tokyo, Japan

Dorsal root ganglion neurons and PC12 cells maintained in the presence of nerve growth factor (NGF), undergo cell death upon its removal. The indolocarbazole alkaloid, K-252a, and a structurally novel analog, CEP1347, promote survival and/or differentiation in a variety of neurons *in vitro* (J.Neurochem.61:201,1993;64:1502,1995). In addition, CEP1347 prevents embryonic and postnatal programmed motoneuron death *in vivo* and prevents the loss of ChAT immunoreactivity in axotomized adult hypoglossal motoneurons (*Soc. Neurosci. Abstr.*1994, 1995). To determine effects of such small "neurotrophic" molecules on cell death thought to be primarily due to apoptotic mechanisms, two *in vitro* systems were employed. In chick embryonic (E9) dorsal root ganglion cultures, K-252a and CEP1347 rescued >50% of the neurons lost after NGF withdrawal (compared to rescue by readdition of NGF). K-252b, a related compound that has no direct effect on the survival and/or differentiation of neurons *in vitro*, showed no protective effect. In PC12 cells, CEP1347 also rescued cells from death after NGF withdrawal, while KT5823, a "non-neurotrophic" analog, had no effect. Actinomycin D partially reduced cell death in both models, suggesting that the cell death may be dependent on RNA synthesis, and a cAMP analog (8-(4-chlorophenylthio)cAMP) prevented cell death in both models. These data support previous findings that CEP1347 prevented cell death *in vitro* and *in vivo*. Furthermore, since cell death in these models is thought to be primarily due to apoptotic mechanisms, CEP1347 may interfere with this process.

704.14

BASIC FIBROBLAST GROWTH FACTOR PREVENTS THE LOW POTASSIUM-INDUCED APOPTOSIS IN THE CULTURED CEREBELLAR GRANULE CELLS. H. Amino*, H. Saito and N. Nishiyama. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo 113, Japan.

Apoptosis, which is characterized morphologically by chromatin condensation, and cytoplasmic and nuclear shrinkage, is one of the pathways of cell death. Cultured mature cerebellar granule cells, isolated from 8-day-old postnatal rat, die by apoptosis when deprived of depolarizing concentration of extracellular potassium (25 mM). We here investigated the effect of basic fibroblast growth factor (bFGF) on this neuronal apoptosis. Exposure of cerebellar granule cells to bFGF (0.01-10 ng/ml) protects cultured cerebellar granule cells (DIV 7), maintained in the presence of physiological concentration of potassium (5 mM), from apoptotic cell death in a concentration dependent manner. This antiapoptotic effect was not mimicked by several other neurotrophic factors, acidic FGF, nerve growth factor and brain-derived neurotrophic factor at the same range of concentration. The trophic action of bFGF was remarkably and significantly blocked by tyrosine kinase inhibitors, herbimycin A and lavendustin A. Protein kinase C inhibitors, staurosporine and H-7, also reversed the protective effect of bFGF, whereas protein kinase A inhibitor, H-89 had no effect. Moreover, neither ionotropic nor metabotropic glutamate receptor antagonists block the antiapoptotic effect of bFGF. These results suggest that bFGF prevents the neuronal apoptosis induced by low potassium in cerebellar granule cells through activation of FGF receptor tyrosine kinase and protein kinase C. It is interesting to speculate that FGF or FGF-like molecules are released in para- or autocrine fashion to prevent the apoptotic cell loss of granule cells in a living rat cerebellum.

704.15

PROTEIN KINASE INHIBITION INDUCES APOPTOSIS IN DEVELOPING RETINAL NEURONS. L. Colombaioni.* Istituto di Neurofisiologia del CNR, 56100 Pisa, ITALY.

Isolated retinas from newborn P_0-P_3 rats are an useful model system for the study of trophic-dependent selection process that occurs during early phases of neuronal development. Culture conditions have been found in which, following serum withdrawal, retinal ganglion cells rapidly undergo death showing morphological and functional characteristics of apoptosis. It is known that neurotrophins promote survival of developing neurons by binding to Trk receptor protein tyrosine kinase, but it is also known that the direct activation of protein kinases often fails to inhibit apoptosis. At the aim of further investigate the role of protein kinase pathway in neuronal death, retinas isolated in serum free medium have been exposed for 3-6 hours to protein kinase inhibitor staurosporine 50-100 nM, a dose considered to inhibit selectively protein kinases C. This treatment remarkably accelerated the time course of ganglion cell death. Under these conditions of protein kinase C inhibition, survival promoting action of serum was lost. These data indicate that prevention of neuronal apoptosis requires activation of protein kinase C pathway. Since other reports indicate that this is not sufficient to prevent neuronal death, it is suggested that simultaneous activation of multiple signaling pathways could be required for neuronal survival.

704.16

INSULIN-LIKE GROWTH FACTOR IS REQUIRED FOR SPINAL MOTONEURON SURVIVAL FOLLOWING SCIATIC NERVE AXOTOMY. S.-F. Pu*, H.-X. Zhuang, D. J. Marsh and D. N. Ishii. Dept. of Biochem. and Mol. Biol. & Dept. of Physiol., Colorado State University, Fort Collins, CO 80523.

The long-term disconnection of motoneurons from muscle targets is known to result in the death of motoneurons in humans. Several lines of evidence, dating from the time of Ramon y Cajal, support the inference that axotomy may deprive motoneurons of soluble neurotrophic substances present both in nerve and muscle. There is a pressing need to identify these substances. We previously showed that insulin-like growth factors (IGFs) are neurotrophic factors, and that IGF mRNAs are increased in nerve and muscle following nerve crush. We tested the inter-related hypotheses that (i) IGF-II can support the survival of postmitotic motoneurons, and (ii) endogenous IGFs normally support the survival of motoneurons. Following sciatic nerve transection in postnatal rats, various substances impregnated in gel foam were applied to the proximal nerve stump. The number of motoneurons were counted in spinal cord sections after 6 days. Recombinant human IGF-II supported the survival of motoneurons, whereas an anti-IGF-II antiserum as well as IGF binding protein-4 increased their death relative to vehicle-treatment ($P < 0.05$). The latter results showed that the activity of endogenous IGF within or close to nerve is required for the survival of injured motoneurons. Although the administration of other exogenous factors may support motoneuron survival, this is the first demonstration that an endogenous neurotrophic factor contributes to survival following nerve damage. These results may explain the observation that the degree of survival is proportional to the length of the remaining nerve stump following transection. IGF is produced in nerve Schwann cells, and more IGF would be available to axons following distal vs. proximal nerve transection. (Supported by American Paralysis Association Grant PA1-9401 and NINDS Grant PO1 NS28323)

704.17

PHARMACOLOGICAL AGENTS THAT MODIFY OXIDATIVE STRESS PROMOTE MOTONEURON SURVIVAL DURING DEVELOPMENT. R.W. Oppenheim*, J. Hong, J. Caldero, C. Milligan, A. Newsome, L.Lloyd and D. Prevette. Wake Forest University Medical School, Winston Salem, NC 27157

Developing spinal motoneurons (MNS) in the chick embryo undergo normal programmed cell death (PCD) in vivo and following trophic factor deprivation in vitro. Previous in vitro studies of other neuronal and non-neuronal cells have suggested that increased oxidative stress may be involved in the pathway leading to PCD. We have begun to examine whether oxidative stress is also involved in the normal PCD of chick MNS in vivo and in vitro. Preliminary studies with one anti-oxidative agent, N-acetylcysteine (NAC), indicates that treatment of chick embryos in ovo during the normal period of MN cell death significantly reduces the number of dying (pyknotic) cells and increases the number of healthy MNS present at the end of the cell death period. Studies are in progress to examine other anti-oxidants and to determine their mode of action (and that of NAC) in preventing the PCD of MNS.

704.18

MECHANISM OF THE OSMOPROTECTIVE EFFECT OF IGF-I IN HUMAN NEUROBLASTOMA CELLS. C. C. Matthews* and E. L. Feldman Dept. of Neurology, Univ. of Michigan. Ann Arbor, MI 48109-0588

We have previously shown that exposure to media made hyperosmotic by the addition of mannitol or NaCl significantly decreased the number of viable SH-SY5Y human neuroblastoma cells within 24 hrs. In contrast, low concentrations of IGF-I preserved cell viability under hyperosmotic conditions. In the current study, we have examined the mechanism of the IGF-I osmoprotective effect.

The IGF-I osmoprotective effect was virtually eliminated by αIR3, a blocking antibody to the type I IGF receptor. Hyperosmotic conditions caused cell cycle arrest of the cells as measured by the rate of ^{3}H-thymidine incorporation and flow cytometry. Addition of 10nM IGF-I protected the cells against hyperosmotic death without stimulating DNA synthesis.

Programmed cell death (PCD) can occur by withdrawal of an essential external signal such as a growth factor. As determined by flow cytometry, several days of serum deprivation in isotonic media did not precipitate PCD. In serum-free hyperosmotic media, PCD was initiated in 40% of cells in 24hrs and 80% in 72hrs. In hyperosmotic media containing 10nM IGF-I, apoptosis occurred in only 12% of cells in 48hrs and 20% in 72hrs. Collectively, our results show that IGF-I acts as a neuronal osmoprotectant by protecting the cells against PCD. These data suggest a potential therapeutic role for IGF-I in diabetic neuropathy or other conditions that lead to hyperosmotic states.

704.19

EFFECTS OF NEUROTROPHIC FACTORS ON THE SURVIVAL OF SENSORY NEURONS FOLLOWING AXOTOMY IN THE NEONATAL MOUSE. L. Li*, A.C. Lo, M. Lei, R.W. Oppenheim and L.J. Houenou
Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

Extensive neuronal death results from peripheral nerve section during the early period of postnatal development in mammals. Recently, it has been reported that a number of neurotrophic factors including BDNF, NT-3, NT-4/5, CNTF, IGF and GDNF promote the survival of axotomized motoneurons in vivo. The present study is aimed at determining whether these neurotrophic factors also rescue axotomized sensory neurons in the neonatal mouse. Our results show that approximately 25% of neurons in the lumbar spinal segment 4 dorsal root ganglion (DRG) died following sciatic nerve section in 5 day old mice, when examined one week after surgery. Several neurotrophic agents including: NGF, BDNF, NT-3, CNTF, IGF-1 were able to promote the survival of axotomized DRG neurons. However, GDNF, previously shown to rescue axotomized motoneurons, was ineffective in rescuing sensory neurons from axotomy induced cell death. Our findings extend previous reports on the effects of neurotrophic agents on axotomized motoneurons and sensory neurons and suggest that some of these agents may be useful in the treatment of diseases and injuries affecting sensory neurons. Supported by Grants from NIH and MDA.

704.20

GDNF AND BDNF PROTECT A CATECHOLAMINERGIC CELL LINE (CATH.a) FROM DOPAMINE INDUCED CELL DEATH. L. Gong*, H. Kulaga, A. Adams, R.J. Wyatt and J.M. Masserano. Neuropsychiatry Branch, NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032.

Glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) promotes survival of dopaminergic neurons in primary cultures and protect these neurons from the neurotoxic effects of 6-OHDA and MPTP. We are using a CNS derived catecholaminergic cell line (CATH.a) as a model to study the effects of growth factors on cell death. Dopamine (DA) (10 - 500 μM) produces a dose dependent cell death (maximal 70%) in CATH.a cells. DA induced cell death is correlated with an increase in intracellular peroxides and is inhibited by catalase, suggesting that cell death is caused by oxidative stress. Treatment of cultures with GDNF or BDNF reduced DA (100 μM) induced cell death from 40% to 20%. Nerve growth factor, fibroblast growth factor and insulin had no protective effect. These results indicate that GDNF and BDNF protect CATH.a cells from the oxidative stress of intracellular peroxides produced by dopamine autoxidation.

705.1

GENESIS OF INTERNEURONS IN THE RAT STRIATUM. A.F. Sadikot*, R. Sasseville, S. Gauthier. Dept. of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, PQ, Canada.

The time of final mitosis (birthdate) of different classes of interneurons in the rat striatum was analyzed using the 5'-bromodeoxyuridine (BrdU) incorporation method. Double-labeling immunohistochemistry allowed simultaneous visualization of BrdU, and either calretinin, parvalbumin, choline acetyltransferase or somatostatin. At the level of the precommissural dorsal striatum, most GABAergic interneurons expressing calretinin are born between E14-E16, whereas most GABAergic interneurons expressing parvalbumin are born between E14-E17. As shown in studies using ^{3}H-thymidine (Semba et al., '88), our studies confirm that the birthdate of the majority of cholinergic and somatostatinergic interneurons at this striatum level is between E13-E15 and E15-E16, respectively. Thus, unlike the patch and matrix projection neurons, whose peak birthdates are E13 and E18, respectively (van der Kooy and Fishell, '87), GABAergic and somatostatinergic interneurons of the dorsal striatum leave the mitotic cycle at an intermediate time period. Further studies are aimed at defining gradients of neurogenesis within the dorsal striatum, and at comparing birthdates of neurons generated in the dorsal or ventral striatum.

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705.2

DIFFERENTIAL EXPRESSION OF GENES PREFERENTIALLY RESTRICTED TO MOTOR NEURONS. **E. F. Salazar-Grueso*, Z. Zhang, and L. Ma. Department of Neurology, University of Chicago, Chicago, IL 60637.**

Molecular processes specific to motor neuron (MN) growth and development are poorly characterized. We are using clonal hybrid cells derived from embryonic mouse spinal cord MN which express traits typical of MN to identify and characterize genes preferentially expressed in MN. cDNAs preferentially expressed in hybrid MN were isolated by differential display reverse transcription polymerase chain reaction and by differential hybridization screening of a hybrid MN cDNA library. cDNAs were sequenced, and submitted for comparison to gene and protein databanks using the BLASTA program. Twenty of 45 (47%) cDNA clones were found to be identical or very homologous to genes previously characterized in mice or rats. Eleven (24%) others had sequence homology to genes previously characterized from humans (9), cattle (1), & fruit fly (1). Twelve (27%) others do not share identity with any currently known genes or proteins. One cDNA sequence was recently identified by others as a G protein β subunit gene. By northern blot we have identified an alternately spliced transcript of this gene which appears to be expressed only in brain and spinal cord. In conclusion, we have used hybrid MN cells to identify novel genes related to MN. One of these, a neural specific gene, appears to be present in MN. Twelve others appear to be novel genes that may have specificity for MN.

Supported by a grant from the Walter Boughton ALS Fund

705.3

FACTORS INFLUENCING THE DEVELOPMENT OF DENDRITE BUNDLES IN THE RAT'S SPINAL CORD.

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Recently, we studied dendrite bundles in motoneuronal pools of muscles, which are involved in posture and locomotion in the rat. Dendrite bundles specifically occur in pools of axial muscles and particular antigravity muscles in extremities. These bundles develop at the age when effective control of posture becomes apparent (from postnatal day 8 [P8] in the pools of the long back muscles and from P14 in the pool of the soleus muscle.

It is not known how this dendritic reorganisation is induced. Possibilities are: [1]by supraspinal afferents; [2]by muscle afferents or [3]by retrograde influences from muscles via their motor nerves. We interrupted the ingrowth of descending projections on P10 by a total spinal transection at Th10-Th11 (N=18) or a hemitransection (N=21). Muscle afferents were interrupted unilaterally at P10 by cutting the dorsal roots of L3-L5 (N=21) and in another group (N=18) we blocked neuromuscular transmission from P3-P12 in the soleus muscle by α-bungarotoxin (in silicon rubber). Dendrite bundles in the pool of the soleus muscle were labeled with Cholera Toxin subunit B at P18, P20 and P30.

Results indicate that blocking neuromuscular transmission retards the development of dendrite bundles while a total transection leads to a decrease in the number of dendrite bundles and dendrites per bundle (although the onset of this reorganisation is normal).Neither sectioning of dorsal roots, nor hemisection of the spinal cord has an effect on this development. It seems that both trophic influences from muscles and supraspinal influences are essential for dendritic reorganization.

705.4

LOCAL INFLUENCES ON REORGANIZATION OF AN IDENTIFIED THORACIC LEG MOTOR NEURON DURING METAMORPHOSIS OF THE MOTH MANDUCA SEXTA. K. S. Kent* and T. M. Bollerman, Dept. Biological Structure & Function, Oregon Health Sciences University, Portland, OR 97201

During metamorphosis of the moth Manduca sexta, thoracic leg motor neurons (MNs) persist from larva to adult but undergo dramatic morphological changes including regression and growth of both dendritic and terminal processes. Previous studies have shown that these central and peripheral changes are under control of ecdysteroid hormone. As part of an on-going study to determine if cell-cell interactions are also involved in shaping these changes, we have examined the regression and growth of terminal processes of an identified leg MN, the femoral extensor MN. In addition, we have used heterochronic mosaic insects in order to determine if local peripheral signals might influence the reorganization of motor terminals independent of the reorganization of central processes. A combination of cobalt and biocytin staining methods, immunocytochemical techniques, and labeling with persistent fluorescent dyes, has demonstrated that regression of motor terminals occurs over the several days preceding pupation as the larval muscle degenerates. Regressed processes remain associated with a remnant of the larval muscle and by three days after pupation, motor terminals demonstrate signs of new growth, displaying filopodial-like processes. Application of a juvenile hormone mimic (methoprene) to the larval legs was used to create mosaics in which larval-like legs with persistent larval leg muscles were retained on a pupal insect. Under these conditions, larval-like motor terminals were retained on the persistent larval leg muscles. In contrast, dendritic regression occurred centrally despite retention of the larval leg. These results indicate that the processes of dendritic regression and terminal regression are not necessarily linked and that local cell-cell interactions influence peripheral changes independent of central changes.

705.5

A TEMPORAL EXAMINATION OF MUSCLE FIBER TYPE IN THE DEVELOPING GUINEA PIG STERNOMASTOID MUSCLE. **W.A.Grow*, P.W.Sheard, M.Duxson, M.B.Laskowski.** WAMI Medical Program, University of Idaho, Moscow, ID 83843.

Due to their size, human muscles have multiple endplate regions, and muscle fibers are usually associated with only one of these regions. Rat muscles, on the other hand, typically contain only one endplate region, and all muscle fibers extend the entire length of the muscle. There are four endplate regions in the guinea pig sternomastoid (SM) muscle, and therefore it is a better model of human muscle development than the rat muscles which have typically been studied. We determined the fiber type composition of the guinea pig SM muscle for both newborns and adults with immunocytochemical staining of fast and slow myosin heavy chain proteins. In the newborn, one edge of the SM muscle consists almost exclusively of fast muscle fibers; by adulthood this region remains exclusively fast but has expanded in size. In the newborn SM slow muscle fibers are always found isolated and surrounded by fast fibers, whereas in the adult SM slow fibers are almost always found in clusters of 2-5 cells. In addition, the number of slow muscle fibers and the total number of muscle fibers are identical for all four endplate regions in the newborn SM; this is also true for the adult SM. Finally, the number of slow muscle fibers and total muscle fibers significantly increases after birth, indicating either transformation of fibers originally with a fast phenotype into slow fibers and/or the generation of new slow muscle fibers. Either possibility could explain the clusters of slow muscle fibers seen in the adult SM. Supported by NIH.

705.6

DEVELOPMENT OF TRIGEMINAL MESENCEPHALIC AND MOTOR NUCLEI IN RELATION TO MASSETER MUSCLE INNERVATION IN MICE . C. G. Widmer* and J. Morris-Wiman. Dept. of Oral and Maxillofacial Surgery and Dept. of Orthodontics, Univ. of Florida, Gainesville, FL 32610.

There has not been a systematic investigation of the spatiotemporal development of nuclei containing sensory (mesencephalic) neurons and motoneurons and their innervation of masticatory muscles. The purpose of this study was to determine the timing of sensory and motor innervation of the masseter muscle and compare this process with the developmental organization of mesencephalic and motor nuclei.

A total of 55 embryos representing gestational days 10.5, 11.5, 12.25, 13.5, 14.5 and 15.5 were obtained from timed-pregnant CD1 mice. DiI or DiA was implanted into the embryonic masseter muscle or masseteric region, into the dorsomedial mesencephalic region, or into maxillary or mandibular molar tooth buds or tooth bud regions. Specimens were maintained at 38°C for 2-8 weeks to facilitate transport of the dye, embedded in gelatin, sectioned at 85 μm using a vibratome, and examined under epifluorescence using a Zeiss Axioplan microscope. Images of labelled cells and their processes were acquired for analyses.

At gd 10.5, 41.6% of the embryos with dye placed in the masseteric region demonstrated labelled cells in the trigeminal motor nucleus while 0% of the mesencephalic nucleus neurons were labelled. Dorsomedial mesencephalic dye placement, however, labelled mesencephalic cell bodies and processes that extended to the motor root, but not into the periphery. At gd 11.5, 100% of the embryos with dye in the masseteric region were found to have labelled cells in the caudal portion of the mesencephalic nucleus and motor nucleus. All embryos older than gd 11.5 with dye in the masseter had labelled cells in the full extent of the mesencephalic and motor nuclei. Dye placement in tooth buds did not label the mesencephalic nucleus at any gestational age examined.

These results demonstrate trigeminal motor innervation to the masseter muscle prior to proprioceptive innervation by mesencephalic neurons. In addition, there is a substantial temporal delay of innervation of the periodontal ligament around the teeth by mesencephalic neurons.

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705.7

EARLY BRAINSTEM-SPINAL SEROTONERGIC AND NORADRENERGIC PROJECTIONS IN THE RAT EMBRYO: DOUBLE LABELING STUDY USING DEXTRAN AMINES AND IMMUNOHISTOCHEMISTRY.

F. Auclair* and R. Marchand. Centre de Recherche en Neurobiologie, Universite Laval, Québec, Canada, G1J 1Z4.

Previous studies done in our laboratory have described the location and discussed the possible identities of neuronal groups projecting to the spinal cord early during the development of the rat embryo. At those stages, the identification of neuronal populations based on their location in the brain and cytology is a hard task. To further characterize those neuronal groups, serotonine (5-HT) and tyrosine hydroxylase (TH) content was assessed combined with retrograde tracing from the spinal cord using dextran amines. The embryos were taken out by cesarean section at the appropriate day of gestation and directly immersed in an artificial oxygenated cerebro-spinal fluid (CSF). The brain was dissected out and a hemisection of the spinal cord was made at the level of the first cervical segment. Fluorescent dextran amines were injected and the preparations were kept in CSF for 12 to 36 hours at 20°C. They were then fixed in paraformaldehyde and cut in a cryostat. Immunohistochemistry was done on the sections mounted on microscope slides. Although many cells near the midline in the caudal brainstem were retrogradely labeled from the spinal cord on day 14 and 15, few neurons were double-labeled after immunohistochemistry against 5-HT. Many cells were also retrogradely labeled from the spinal cord in the rostral lateral pons on day 14, 16 and 18. Again, few neurons were double-labeled after immunohistochemistry against TH on day 14 and 16, but a significant number was on day 18 in locus coeruleus and subcoeruleus. A large number of the retrogradely labeled neurons in the rostral lateral pons were located just medial to the TH-positive neurons, in a region that could be associated with Barrington's nucleus of the adult. In conclusion, although 5-HT-positive neurons in caudal raphe nuclei become immunoreactive around E13, a large number has not reached spinal cord levels at E15. Also, many neurons in the rostral lateral pons become TH-positive around E14 but only a few have reached spinal cord levels by E16.

705.8

SEROTONIN INCREASES THE EXCITABILITY OF EMBRYONIC CHICK MOTONEURONS RECORDED FROM A SPINAL CORD SLICE PREPARATION. T. Hayashi, B. Mendelson, K.D. Phelan and E. Garcia-Rill*. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Intracellular recordings were obtained from motoneurons in an embryonic chick spinal cord slice preparation over a series of developmental stages (E12-E18). These time points bracket the critical period of development (E14) in which the ability of the spinal cord to recover from injury is lost. Transverse slices (<1 mm thick) were isolated from lumbosacral segments 1-3. Ventral roots were maintained for stimulation. Motoneurons were identified by antidromic activation. Bath application of serotonin (5-HT; 50-100 µM) was used to determine its effect on rheobase, action potential threshold, afterhyperpolarization and some firing frequency properties. Rheobase was determined as the amplitude of the current pulse of >300 msec duration at 1 Hz that produced an action potential during 50% of the pulses. The absolute membrane potential at the onset of the action potential was defined as the action potential threshold. To analyze firing frequency, depolarizing pulses of 1 sec duration at 0.2 Hz were applied at 0.1 nA increments. Serotonin induced a membrane hyperpolarization in the majority of motoneurons at E12 and a depolarization in all of the cells recorded at E18. However, at all stages examined, the firing frequency of the vast majority of motoneurons was increased during the serotonin administration. Rheobase and membrane potential at action potential threshold were decreased following 5-HT administration. This 5-HT effect was greater in E18 compared to E12 cells. The peak amplitude of the afterhyperpolarization was also decreased during the serotonin administration. These results indicate that 5-HT administration increases the level of excitability of motoneurons at all stages examined. Supported by NSF grant RII 8922108, USPHS grants NS20246 and AA09205.

705.9

RELATIVE CONTRIBUTIONS OF POTASSIUM CONDUCTANCES AND SYNAPTIC INPUT IN DETERMINING INPUT RESISTANCE OF DEVELOPING BRAINSTEM MOTONEURONS. T.Hodgson and W. Cameron. Dept. of Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Between postnatal week 1 and 2, the mean input resistance of rat genioglossal motoneurons drops 50% without an increase in total membrane surface area. To investigate the mechanism underlying this decrease, intracellular recordings were made from genioglossal motoneurons in an *in vitro* brainstem slice preparation from 6-7 day, 13-15 day and 18-25 day old rat pups. Input resistance was measured from the linear portion of the voltage response. Perfusion with high Mg^{2+} (6mM) ACSF increased input resistance compared to control (mean = 10% in 6-7 day olds; mean = 2% in 20-25 day olds). This suggests a decreasing contribution by calcium-dependent synaptic release to motoneuron input resistance. Intracellular Cs^{+} injection also produced an increase in input resistance (mean = 11% in 6-7 day olds; mean = 27% in 20-25 day olds), reflecting the increasing involvement of potassium conductances in input resistance over development. In order to determine the change in the relative contribution of synaptic activity and potassium channels during the first weeks of postnatal life, we measured input resistance in: control ACSF(2mM Ca^{2+} /2mM Mg^{2+}), high magnesium (6 mM Mg^{2+}/ 1mM Ca^{2+}) and high magnesium/TEA (6mM Mg^{2+}/ 1mM Ca^{2+}/ 20mM TEA). Mg^{2+} made a smaller contribution than TEA to the total increase in input resistance in all age groups. In addition, the relative contribution of Mg^{2+} versus TEA changed with increasing postnatal age: 25% Mg/75% TEA in 6-7 day olds, 15% Mg/85% TEA in 13-15 day olds, and 5% Mg/95% TEA in 20-25 day old rat pups. Supported by HD 22703.

705.10

MYELOGENESIS OF THE VENTRAL AND DORSAL ROOT AT THE BRACHIAL AND LUMBOSACRAL ENLARGEMENTS OF THE OPOSSUM, *MONODELPHIS DOMESTICA*. H. Leblond* and T. Cabana. Sciences Biologiques, Université de Montréal, C.P. 6128, Succ. Centre-Ville, Montréal, Canada, H3C 3J7.

The opossum is born more immature than any placental mammal. At birth, its forelimbs are sufficiantly developed to perform rhythmic, alternate movements, but the hindlimbs are immobile. The latter do not begin to move until about postnatal day (P)10. In light microscopy, we have investigated myelogenesis of the ventral (VR) and dorsal (DR) roots of spinal segments that innervate the forelimb (C8) and hindlimb (L4), selected because they virtually contain exclusively somatic fibers. The tissue was fixed with a modified Karnovsky's fixative, post-fixed with 2% OsO4, embedded in resin, the roots were cut transversely at 1.5µm, stained with toluidine blue and photographed. In the P1 opossum, both roots at both segments are unmyelinated. At C8, the first myelinated fibers are seen in the VR at P15 and in the DR at P17. At L4, myelinated fibers are first seen in the VR at P17 and in the DR at P22. At P25, the C8 VR contains about 70% of its adult number of myelinated fibers but the DR only 30%; the L4 VR contains 26% and the DR 10% of their adult numbers of myelinated fibers. However, at P25 myelinated fibers are comprised of a single population in terms of diameter, myelin sheath included. It is only at P50 that they can be separated into distinct populations: alpha and gamma for the VRs and Types I, II and III for the DRs. Also at P50, the total number of myelinated fibers in both L4 roots is greater than in the adult. These data will be correlated with the ontogeny of motor behaviors. (Supported by NSERC)

705.11

AUGMENTED KINESTHETIC FEEDBACK PROMOTES MOTOR LEARNING IN THE RAT FETUS. S. R. Robinson*. Department of Psychology, University of Iowa, Iowa City IA 52242.

The capacity of the rat fetus to selectively alter its motor activity in response to kinesthetic feedback was evaluated in a model of fetal motor learning. After surgical preparation of the pregnant rat to permit fetal observation, E20 rat fetuses were fitted with a fine thread tied to both rearlimbs. With this yoking procedure, it was expected that an active movement by one limb would result in passive movement of the other limb, thereby producing additional kinesthetic feedback that could facilitate interlimb coordination. Experimental subjects (YOKED) were tethered for 30 min, after which the connecting thread was cut and the subject observed for another 30 min. Control subjects were either fitted with a thread that was immediately cut (UNYOKED) or received no treatment (NT) and were observed without interruption for 60 min. YOKED subjects showed a 6- to 8-fold increase in the frequency of synchronous movements of the left and right rearlimbs. No change in synchronous rearlimb activity was evident in the UNYOKED or NT groups. The observed increase in synchronous movements was not expressed immediately, but emerged gradually over the 30-min period in which the limbs were physically coupled, and persisted for 20-25 min after the yoke was cut. Other experiments revealed that the effectiveness of the yoking procedure varied with the elasticity of the connecting thread; a stiff linkage was more effective than an elastic thread in promoting interlimb synchrony. A similar increase in synchronous forelimb movement was promoted when the yoke was applied to the two forelimbs. These data provide evidence that the E20 rat fetus possesses a functional kinesthetic sense, and that the fetus can alter the organization of motor behavior to adjust to varying conditions of biomechanical constraint.

705.12

HUMAN FETAL AND NEONATAL MOVEMENT PATTERNS AND EFFECTS OF CNS ABNORMALITY. C. R. Almli*, N. M. Mohr, R. Ball, and L. Bernhard. Developmental Neuropsychobiology Lab., Washington University Medical School, St. Louis, MO 63108.

Little is known about development of spontaneous movement patterns (autogenic movement patterns, AMP) of human fetuses and neonates, e.g., how fetal AMP may change following birth (neonatal AMP), and, how fetal and neonatal AMP may be altered by prenatally-identified CNS abnormality. Quantitative analysis of AMP determined: (1) continuity/dis-continuity of fetal-to-neonatal AMP in normal subjects, (2) how fetal/neonatal AMP may be altered by prenatal CNS abnormality (e.g., myelomeningocele)

AMP video tapings were obtained for fetuses (third trimester: ultrasonography) and for neonates (birth and 6 weeks postnatal). Leg AMP of fetuses/neonates were quantified with a computer-video movement analysis system.

Results for numbers and periodicity of leg movements indicates: antenatal-to-postnatal AMP continuity for normal fetal/neonatal subjects, and altered fetal/neonatal AMP for CNS abnormality subjects. Results suggest that fetal/neonatal AMP, which is under control of central pattern generators, is disrupted by prenatal CNS abnormalities, and that fetal/neonatal AMP may not be as strongly influenced by environmental factors (intra- to extra-uterine transitions) as previously thought.

705.13

GROOMING DEVELOPMENT AND ACTIVATION IN WEAVER MUTANT MICE. V.J. Bolivar*, W. Danilchuk and J.C. Fentress. Psychology Department, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1.

Weaver (*wv/wv*) mice have well specified nigrostriatal dopaminergic and cerebellar defects. Quantitative analyses of behavioral development in these animals have not previously been pursued. We examined grooming in *wv/wv* pups and littermate controls before and after a 30 second swim period. Deficiencies in grooming of *wv/wv* pups are context and age dependent. *Wv/wv* spend less time grooming than controls; however, after day 13 postpartum during the post-swim period mutants attained grooming levels of pre-swim controls. *Wv/wv* pups also showed, from day 15, an increase in number of grooming bouts post-swim. This reflects previous activation by swimming. Our data suggest activational effects can be separated from balance, postural or sequencing problems. Although controls displayed longer bouts than mutants overall, during the post-swim period (day 13 and older) *wv/wv* pups produced bouts as long as pre-swim controls. Strokes used by *wv/wv* pups tended to cluster around the early grooming sequence phase and some later strokes were never used by the mutants. These results indicate the importance of examining behavioral ontogeny under different conditions, as well as the value of using multiple measures when examining action sequences. Mutant mouse research holds much promise in linking cellular, circuit and behavioral levels of organization.

705.14

SNAP-25 ISOFORM mRNA EXPRESSION IN SPINAL MOTONEURONS AFTER NERVE INJURY. G. Jacobsson, F. Piehl*, X. Zhang, I.C. Bark and B. Meister. *Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, and Department of Developmental Biology, Uppsala University, Sweden.*

Synaptosomal-associated protein of 25 kDa (SNAP-25) is a protein involved in the molecular regulation of exocytosis. SNAP-25 is located at the presynaptic plasma membrane and is a component of the 20S multiprotein complex that mediates vesicle docking and fusion. The protein exists in two isoforms, which arise from alternative splicing of exon 5. In order to study the plasticity in SNAP-25 gene expression, we have axotomized the sciatic nerve and studied SNAP-25 mRNA levels in spinal motoneurons using *in situ* hybridization and isoform-specific oligonucleotide probes. In all animals, SNAP-25a mRNA was detected in the nucleus of motoneurons, whereas SNAP-25b mRNA was present in the cytoplasm. After axotomy, the levels of SNAP-25a and SNAP-25b mRNA decreased in motoneurons belonging to the sciatic pool. Measurements of the grain density over cells on the lesioned side as compared to the unlesioned side revealed a significant decrease after 2 days. The maximum decrease was detected at 7 days (62% for SNAP-25a and 67% for SNAP-25b), while levels had slightly recovered by 14 and 28 days. Ventral root avulsion produced a similar decrease in SNAP-25 mRNA levels in lesioned cells, indicating that the decrease in SNAP-25 mRNA is due to the axonal severance in itself. The results suggest that SNAP-25a and SNAP-25b mRNA have different localization within the cell and that SNAP-25 mRNA is down-regulated in specific populations of spinal motoneurons after nerve injury.

705.15

FACTORS RESPONSIBLE FOR POOR FUNCTIONAL RECOVERY AFTER PERIPHERAL NERVE LESIONS. Neil Tyreman, Mukaila Raji and Tessa Gordon*. Department of Pharmacology and Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada T6G 2S2.

Even primary nerve repair may be associated with dismal functional recovery when long delays are incurred between the peripheral nerve injury and target reinnervation. In this study, we have systematically and independently controlled the duration of axotomy (X), denervation of the distal nerve sheaths (SH) and muscle denervation (M) to determine the relative contribution of each factor to functional recovery. In rats, the posterior tibial nerve (TIB) was cross-sutured to the CP nerve via a contralateral common peroneal (CP) nerve graft (10-15mm) to reinnervate tibialis anterior (TA) muscle, either immediately or after prolonging TIB axotomy, CP graft or TA denervation. At least 6 months after graft surgery, number and size of TA motor units were determined as measures of number of regenerated nerves and numbers of muscle fibers reinnervated per motoneuron. An average of 99.4±15.3 motoneurons innervated TA after immediate grafting. In all cases where nerve repair was delayed, the number decreased as a function of delay between AX, SH or D and nerve repair, reaching an asymptotic value of 30.2±3.6, 41.4±4.1 and 13.7±3.4 at 300 days.

In the light of our previous findings that deterioration of intramuscular nerve sheaths is the primary factor limiting reinnervation of long-term denervated muscle (Fu & Gordon, J. Neuroscience, in press), these findings indicate that the growth response of AX motoneurons and the growth environment of denervated nerve sheaths deteriorates with time. The better recovery after prolonged graft denervation than after prolonged muscle denervation indicates that migration of Schwann cells from the "fresh" nerves into the "old graft" may have facilitated regeneration. We are presently evaluating the molecular basis for the reduced growth response of long-term axotomized nerves and denervated nerve sheaths (see You et al. abstract).

705.16

ACCUMULATION OF NEUROFILAMENTS CAUSES DENDRITIC ATTRITION IN MOTOR NEURONS. J.-M. Kong, D.W. Cleveland+, and Z.-S. Xu*. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545; +Ludwig Institute for Cancer Research and Department of Neuroscience, UCSD, La Jolla, CA 92093.

Accumulation of neurofilaments (NFs) in motor neurons has been widely observed in human motor neuron disease, of which the most common forms are amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). In transgenic mice, severe accumulation of NFs induced by forced over-expression of either NF-L or NF-H leads to loss of motor function, resulting in paralysis, skeletal muscle atrophy, and finally death (Xu et al, 1993; Cote et al, 1993). To further understand how NF accumulation causes motor neuron dysfunction, we have examined the structure of neuromuscular junction by EM and motor neuron morphology by Golgi staining in the NF-L transgenic mice. Except for the increased NF content in nerve terminals, the neuromuscular junction remains intact. However, a quantitative measurement of the spinal cord motor neurons reveals a significant shortening in the length of their dendrites (by more than two fold in comparison with the wild type), leading to a reduced density of dendrites, a shrunken area coverage by the dendrites, and a loss of distal dendrites. These changes could cause a reduction in the number of synapses contacting the motor neurons and consequently lead to motor neuron dysfunction.

705.17

STUDIES ON SLOW AND FAST COMPONENTS OF AXONAL TRANSPORT IN MICE LACKING THE NEUROFILAMENT LIGHT (NFL) GENE. S. Couillard-Després, Q. Zhu, D. E. Playford* and J.-P. Julien. Centre for research in Neuroscience of McGill University, The Montreal General Hospital Research Institute, 1650 Cedar ave., Montreal, Quebec, Canada, H3G 1A4.

The neurofilament triplet proteins form the 10 nm filaments found in almost all neuronal cells and are the major constituents of the cytoskeleton in these cells. The presence of the three components (neurofilament light, medium and heavy subunits: NFL, NFM and NFH) is required for a proper polymerization. Our laboratory recently produced a mouse line lacking the NFL gene. In the absence of NFL, expression of the two other subunits, NFM and NFH, in the nervous system is down regulated. Our data reveals the existence of a compensatory mechanism resulting in the up-regulation of tubulin and microtubule-associated proteins. To study axonal transport, ^{35}S-methionine was injected in the ventral horn of the spinal cord (between L2 and L3) of knock-out and control mice. The mice were sacrificed after different time intervals following injection. The sciatic nerves were dissected and divided into 5 mm segments. Protein was extracted from each segment, fractioned on SDS-PAGE, and analyzed by fluorography. Our preliminary results reveal a dramatic increase in the rate of axonal transport of tubulin in the NFL knock-out mice.

706.1

LAMINAR DISTRIBUTION OF GLUTAMATE RECEPTORS IN DEVELOPING RAT VISUAL CORTEX. B. Gordon*, L.N. Fu and T. Lissman, Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403

We studied the development of glutamate binding sites in the visual cortex of Long-Evans rats. We used quantitative autoradiography to describe the development of [^{3}H]MK-801 binding sites (an open channel ligand for NMDA receptors), [^{3}H]kainate binding sites, and [^{3}H]AMPA binding sites. As a non-glutamate control we used [^{3}H]muscimol, a ligand for $GABA_A$ receptors. Binding of each ligand was measured at 14, 18, 26, 39 days (d) and in adults (>90 d). At 14d [^{3}H]MK-801 binding was similar across all layers, with a slight peak in layers 2/3 and 4. In older animals [^{3}H]MK-801 binding peaked in layers 2/3 and declined in the deeper layers. Binding in layers 5 and 6 was greater in both 26d and 39d animals than in adults. Thus, MK-801 binding in the deeper layers of the rat visual cortex appears maximal around 30d, the approximate peak of the critical period (Fagiolini et al. 1994). [^{3}H]Kainate binding was maximal in layers 5 and 6 and was not age dependent. AMPA binding did not vary dramatically by layer, although it decreased slightly from layer 2 to layer 6. In layers 4-6, AMPA binding was lower in adults than in 26d or 39d animals. Except at 14d muscimol binding was greatest in layers 2/3 and 4. Here muscimol binding was greater at 26d than in adults. These results suggest that NMDA receptors are involved in plasticity in the rat. AMPA binding sites may also be involved. The precise role of these binding sites requires better definition of the rat critical period. The present results in rat contrast somewhat with results we reported previously for cat (cf. Gordon et al., 1994). (Supported by NEI grant EY04050 to BG).

706.2

COLOCALIZATION OF NMDA-R1 mRNA AND GLU-R1 IMMUNOREACTIVITY IN THE RAT VISUAL CORTEX. Z. Cao*, B. Gordon, and M. Lickey. Inst. of Neurosc., Univ. of Oregon, Eugene, OR 97403.

NMDA receptors may mediate activity dependent plasticity in the visual cortex during development (for review see Fox and Zahs, 1994). In rats, maximal visual plasticity occurs at approximately 30 days of age (Fagiolini et al., 1994). In the visual cortex of Long-Evans rats, we studied NMDA receptor gene NMDA-R1 with in situ hybridization and AMPA receptor protein Glu-R1 with immunohistochemistry. (The NMDA-R1 cDNA was a gift from Dr.Koki Moriyoshi, subcloned by Dr.Gilbert Burns). Glu-R1 immunoreactivity was similar in 30 day and 90 day animals. It was present in all layers but was highest in layer 1 and lowest in layer 4. The level of NMDA-R1 gene expression was much higher at 30 days than at 90 days. At both ages NMDA-R1 expression was maximal in the top half of layers 2/3. Double labeled cells were found primarily in 30 day animals. The majority of the cells in 30 day animals were double labeled and double labeled cells were found in all layers except layer 1. These data show that NMDA-R1 expression is greater at the height of the critical period than in adulthood. This result is consistent with the hypothesis that NMDA receptors are involved in visual cortex plasticity in the rat. The role of the Glu-R1 protein is less clear, since the abundance of this protein does not appear to decline in association with declining plasticity. In future studies we plan to examine additional age groups and subunits. Supported by NEI grant EY04050 to Barbara Gordon.

706.3

REGIONAL AND LAMINAR CHANGES IN SYNAPTIC LOCALIZATION OF NMDA RECEPTOR SUBUNIT SPLICE VARIANT NR1 IN RAT VISUAL CORTEX AND HIPPOCAMPUS. R. R. Johnson*, X.-P. Jiang, and A. Burkhalter, Dept. Anatomy & Neurobiology, Wash. Univ. School of Medicine, St. Louis, MO 63110.

Regional and laminar distribution of the NR1 subunit of the NMDA receptor was examined at the light (LM) and electron microscopic (EM) levels using a new antiserum directed against a differentially spliced C-terminal exon (NR1-C1; Sheng et al., 1994). This exon is expressed in a restricted number of NR1 splice variants (Sugihara et al., 1992; Hollman et al., 1993) and is a major substrate for posttranslational modification by phosphorylation (Tingley et al., 1993). The most striking result was that the pattern of NR1-C1 immunoreactivity in both the hippocampus and visual cortex was more restricted than that previously shown using other NR1-specific antibodies (Huntley et al., 1994; Petralia et al., 1994; Siegel et al., 1994), and did not label cells in CA3, dentate gyrus and subicular regions of the hippocampus and in layer 4 of the visual cortex. Regional and laminar differences in synaptic NR-C1localization were confirmed by quantitative EM.

Surprisingly, in addition to abundant postsynaptic staining, immunoreactivity was found in >40% of axon terminals in the dorsal subiculum, but in only a very small percentage (<1%) of terminals in visual cortex. This strongly suggests that presynaptic NMDA receptors play a major role in neuronal processing of hippocampal output through the subiculum, but have a relatively minor role in V1.

In sharp contrast to the pattern in the adult, there was relatively little regional and laminar variation in NR1-C1 immunoreactivity in the developing brain (PND 11). Interestingly, NR1-C1 splice variants were present in cortical layer 4 as well as in CA3, dentate gyrus, and subiculum at the early time point but were largely excluded from these structures in the adult. This suggests that NR1-C1 splice variants may play a role in determining the critical period for visual cortical layer 4. Continued expression within upper and lower layers of the cortex and in CA1 of the hippocampus in the adult could provide a substrate for plasticity in corticocortical connections and Schaffer collateral synapses beyond the critical period.

706.4

DIFFERENTIAL DISTRIBUTION OF TWO GLUTAMATE DECARBOXYLASES (GAD67 AND GAD65) IN THE NEWBORN AND ADULT CAT VISUAL CORTEX. Y. Guo, I.V. Kaplan, N.G.F. Cooper and G.D. Mower*. Univ. of Louisville Sch. of Med., Louisville, KY 40292.

The identification of two forms of glutamate decarboxylase (GAD67, GAD65), the synthetic enzyme for GABA, raises the possibility that they may play different roles in the development of mature GABAergic circuitry. We have used antibodies to compare GAD67, GAD65, and GABA positive neurons in neonatal (4-6 days) and adult cat visual cortex. In adults, GABA and GAD67 containing neurons were found in all cortical layers but little cell body staining was seen with the antibody to GAD65. Higher power observation revealed a complementary intracellular distribution of GAD67 vs GAD65. GAD67 was found mainly in cell bodies and was present to a lesser degree in puncta (presumptive synaptic terminals) than GAD65. GAD65 was faint in cell bodies but labeled more puncta than GAD67. In neonates, GABA and GAD67 were densest in two distinct bands, one superficial (Layer I), another deep (Layer VI/Subplate). Unlike in adults, GAD65 positive cell bodies were clearly evident in neonates and distributed similarly to, but less frequently than, GABA and GAD67. Higher power observation again revealed that the density of GAD65-positive punctate structures was higher than that of GAD67-punctate structures. The distinct intracellular localization of the two isoforms in adult cat visual cortex is consistent with the notion that GAD67 (holoGAD) provides the basal pool of GABA and GAD65 (apoGAD) is specialized to respond to short-term increases in demand for GABA in axon terminals. That the intracellular and laminar distribution of both isoforms of GAD and of GABA differs between neonatal and adult VC suggests that these two forms of GAD may be differentially involved in two distinct aspects of postnatal maturation of visual cortical GABA circuitry: dying off of embryonic cells and differentiation of the adult GABA system.

706.5

DEVELOPMENTAL EXPRESSION OF THE IMMEDIATE EARLY GENE EGR-1 MIRRORS THE CRITICAL PERIOD IN CAT VISUAL CORTEX. I. V. Kaplan, Y. Guo, K. Klueber* and G. D. Mower. Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292.

The aim of this study was to determine the postnatal developmental profile of EGR-1 protein expression across the layers of cat visual cortex and relate it to the state of visual cortical development and plasticity. Cats at various postnatal ages (0.5 week, 1 week, 2.5 weeks, 5 weeks, 10 weeks, 20 weeks, and older than 1 year) were used. Coronal sections from visual cortex were processed for immunohistochemistry with avidin-biotin visualization. In very young animals (0.5 weeks), EGR-1 positive cells were restricted to deep cortical layers (layer VI/Subplate). With increasing age, EGR-1 immunoreactivity spread across layers of the visual cortex in inside-outside manner, and by 5 weeks of age, EGR-1 protein was highly expressed in all layers of the visual cortex. In addition, an intensely stained band of labeled cells in the lowest portion of layer IV appeared at 1 week of age and was present in all older animals. EGR-1 expression remained high until approximately 10 weeks of age and then gradually began to decline in layer IV with little change in supra- and infragranular layers. Therefore, in adult animals, EGR-1 positive neurons were located predominantly in the layers above and below layer IV.

This pattern of EGR-1 expression in developing cat visual cortex exhibits both temporal and laminar similarities with the development of visual cortical connectivity, with the development of orientation selective receptive field properties, and with the level of visual cortical plasticity, suggesting an involvement of EGR-1 expression in these processes.

706.6

LAMINAR PATTERNS OF $GABA_A$ RECEPTOR EXPRESSION IN FERRET PRIMARY VISUAL CORTEX ARE ESTABLISHED PRIOR TO CORTICAL INVASION BY GENICULATE AFFERENTS. A.L.Smith and I.D.Thompson*, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

Quantitative *in vitro* autoradiography has been employed to monitor the expression patterns of $GABA_A$ receptor proteins in the primary visual cortex of ferrets throughout postnatal development. [^{3}H]Musicimol, a $GABA_A$ receptor agonist, and [^{3}H]flunitrazepam, an agonist at the benzodiazepine modulatory site, revealed very similar expression patterns in cryostat sections. Presumptive primary visual cortex was identified by transneuronal axoplasmic tracing of geniculate axon terminals using intraocular injections of wheatgerm agglutinin conjugated to horseradish peroxidase (4%, 2-4µl) and the development of the cortical plate was monitored by neuronal birthdating using 5-bromo-2'-deoxyuridine immunocytochemistry.

$GABA_A$ receptor density within primary visual cortex doubles in the first postnatal week, after which time layer-specific laminar distribution patterns are observed. From postnatal day 8 (P8), when migrating layer IV neurons are arriving in the cortical plate, until adulthood receptor density in layers I and IV exceeds that found in the infragranular layers or layers II/III ($P<0.001$). Receptor protein expression in all layers continues to increase throughout development, increases in layers I and IV preceding those in other layers, reaching a maximum a month after eye opening and then decreasing slightly after P85. Unlike EAA receptors (Smith & Thompson, *J. Physiol.* 485:8P) changes in $GABA_A$ receptor distribution do not appear to be linked with changes in the state of the geniculate fibres and preliminary evidence indicates that disruption of the geniculocortical pathway does not affect the early patterning of $GABA_A$ receptor expression.

706.7

CHANGING DISTRIBUTION OF NMDAR1 IN THE DEVELOPING VISUAL CORTEX S. M. Catalano*, C. K. Chang and C. J. Shatz, HHMI and Dept. of Molecular and Cell Biology, University of California, Berkeley, 94720

NMDA receptors are thought to play an essential role in the development of the visual cortex. We have examined the distribution of the NMDAR1 receptor subunit protein in the ferret using 3 different antibodies: two are raised against overlapping, C-terminal, alternatively spliced domains (Ab1 & 2), and the third is raised against a separate, non-alternatively spliced domain (mAb3). At embryonic day 35 (birth = E 41), all 3 antibodies stain fibers within marginal zone (future layer 1), cells in the cortical plate, and subplate neurons (located within future white matter); the subplate neurons are stained most intensely. There was no detectable immunostaining of migrating cells in the intermediate zone. By P 3, cells within layer 6 stain as intensely as the subplate neurons. In addition mAb3, but not the other antibodies, stains a band of horizontally oriented fibers within the intermediate zone. By the 7th postnatal week, when ocular dominance columns have begun to form in cortical layer 4, staining of layer 1 and the white matter (subplate neurons) is undetectable with all 3 antibodies. Within the cortical layers, mAb3 stains layers 4 and 2/3 more intensely than layers 5 and 6, whereas the other 2 antibodies stain neurons in all layers with equal intensity. In the adult, the mAb3 antibody stains cells in layers 2/3 more intensely than those in layers 4-6. These observations indicate that 1) the earliest cells of the neocortex to be stained are subplate neurons, and 2) during the period of ocular dominance column formation, there is a relative decrease in the R1 subunit in layer 4 as detected by the antibody that recognizes an epitope which is not alternatively spliced (mAb3). These results are consistent with a role for NMDA receptors not only during the critical period, but also far earlier in visual cortical development. Supported by grants from the NEI to CJS (EY02858) and SMC (EY06491). We thank T. Dawson for the generous gift of Ab1.

706.8

SUBCELLULAR REDISTRIBUTION OF NMDA RECEPTORS IN DEVELOPING VISUAL CORTICES OF CATS. C-G Go*, Chiye Aoki, N. Nurbhai, C. Venkatesan, X.-Z. Song & Ted M. Dawson+. Ctr for Neural Sci., New York Univ., NYC, NY 10003 & +Dept. Neurol. & Neurosci., The Johns Hopkins Univ. Med. Schl., Baltimore, MD.

Earlier electrophysiological studies have shown that, during the postnatal period spanning the critical period, NMDA receptors in the visual cortex undergo alterations in their laminar distribution and are more sensitive to blockade by NMDA-receptor antagonists. Using a previously described antiserum directed against the NMDA-R1 subunit, we determined whether these pharmacological changes correlate with changes in the cellular and subcellular distributions of the receptor. During the first three postnatal weeks, light microscopy showed prominent staining of a small subset of neurons that were scattered throughout the cortical thickness, including layer 6B. These neurons exhibited non-pyramidal perikarya and varicose dendritic processes which were studded with intensely immunoreactive patches. In addition, fine beaded processes, most likely axonal, were prominent in the white matter. In contrast, tissue obtained from older animals exhibited a more uniform distribution of punctate labeling in the neuropil. Electron microscopy revealed that these consist of immunoreactivity in small patches along plasma membranes of dendrites, axons and terminals. These results indicate that the NMDA-R1 subunit undergoes dramatic redistribution during the critical period for developmental plasticity. Supported by NEI-EY08055, NSF Presidential Faculty Fllwshp RCD 92-53750, NINDS NS30944-01 & HFSP RG-16/93.

706.9

SYNAPTIC VESICLE PROTEIN IMMUNOREACTIVITY DURING DEVELOPMENT IN MACAQUE MONKEY PRIMARY VISUAL CORTEX. T. Yoshioka* & S.H.C. Hendry. Krieger Mind/Brain Inst. & Dept. of Neuroscience, Johns Hopkins University, Baltimore, MD 21218

Changes in synaptic activity are essential in the functional and anatomical development of mammalian neocortex. Such synaptic activity is influenced by intrinsic and extrinsic neuronal input in different laminae during development. To assess the pattern of laminar-specific events in the development of synapses in macaque primary visual cortex (V1), we have examined the expression of synaptophysin, an integral membrane protein of synaptic vesicles, in the fetal and infant macaques. At embryonic day E135 (gestation = 165 days), immunostaining of synaptophysin revealed distinct laminar boundaries, with intense punctate immunoreactivity in layers 4A, 4Cα, and the bottom of 4Cβ through 5. Immunostaining in layer 4A showed clear evidence of a honeycomb or lattice, even though no pattern was evident in the pale cytochrome oxidase staining of layer 4A at this age. A similar irregular honeycomb pattern was also evident in deep layer 5. The laminar pattern seen at E135 continued to be visible in later age, but became less laminar-specific immunostaining in postnatal tissues. These observations suggest that the maturation of synapses occurs in a laminar specific manner in macaque V1. (Supported by NIH grant EY06432. We thank Dr. Anita Hendrickson for providing fetal and neonatal tissue.)

706.10

SPONTANEOUS ACTIVITY OF NEURONS IN ORGANOTYPIC CULTURES OF RAT NEOCORTEX DURING DEVELOPMENT *IN VITRO*. D.Echevarria* and K.Albus, Neurobiology Groups, Max Planck Institute for Biophysical Chemistry, 37018 Göttingen, FRG.

Neurons in organotypic cultures (OTCs) of rat cortex may develop highly dependent firing patterns, including sterotyped burst pattern (1). We wondered whether in neocortical OTCs spontaneous bursting activity displays a progessive time-dependent onset as it has been demonstrated for neurons in OTCs of hippocampus. Action potentials were recorded extracellularly from 640 neurons in 102 OTCs (from 3 - 9 days old rats, roller tube technique) of rat occipital cortex between 4 and 64 days in vitro (DIV). Neurons were divided operationally into 4 classes: 1) Not spontaneously active (NonSp) neurons, detected only by electrical stimulation; 2) Spontaneously active, regularly firing (SpR) neurons; 3) Spontaneously active, bursting (SpB) neurons; 4) Spontaneously active, mixed (SpM) neurons, i.e. with both SpR and SpB activity. In OTCs 4 DIV all activity classes were present. The proportions of activity classes changed during development *in vitro*. From the first to the second week the proportions of NonSp neurons decreased (from about 60% to about 30%) and that of spontaneously active increased accordingly. From thereon no systematic change occured until the end of the 2nd month. During development in vitro a slight (not significant) upward trend in average firing rate, intraburst firing rate and burst duration was found. A spatial and temporal analysis of firing patterns by means of double recordings revealed a high degree of burst synchronicity across OTCs with multiple sources for triggering individual bursts. A progressive time dependent onset of bursting activity was not seen. Rather it was found that SpB neurons, in particular those displaying longer lasting bursts, were present from the 4th DIV on in OTCs treated with more than average mechanical manipulation during preparation.

(1) Gutnick et al.*E.B.R* .76:131-140 (1989); McBain et al. *J.N.Meth.*27:35-49 (1989). Supported by A.v.Humbolt SPAK 0025 and Gobierno Vasco BIF94.046.

706.11

DEVELOPMENTAL CHANGES IN EVOKED SPATIAL ACTIVITY PATTERNS IN CORTICAL SLICE CULTURES. Y. Jimbo[1], P.J. Charlety[1], H.Kamioka[1], H.P.C. Robinson*[2], and A. Kawana[1]. [1]NTT Basic Res.Labs, 3-1 Morinosato Wakamiya, Atsugi-shi, Kanagawa 243-01,JAPAN. [2]Physiol. Lab, Univ. Cambridge, U. K.

Spontaneous synchronized activity is believed to play a role in the refinement of connections in the developing nervous system. Developmental activity changes in dissociated cortical cultures have been described previously (Charlety *et al., Soc. Neurosci.* 1994, 534.9). Here we have mapped developmental changes in the spatial profile of evoked activity in cultured cortical slices. P2 rat cortical slices were fixed by gentle centrifugation onto substrates containing an array of 64 embedded electrodes, and cultured. After 5 days in vitro (DIV), stimulation (1.5 V, 100 μs, every 5 s) was applied individually through 16 different substrate electectrodes and the evoked responses were recorded at 8 sites. In normal medium at 5 DIV, each stimulus evoked a burst of action potentials of about 200 ms duration whose structure showed evidence of summed activity from multiple neurons in the vicinity of the recording electrode. Latencies varied with the site of recording, revealing an anisotropic propagation of the burst activity through the slice from the site of stimulation. At 9 DIV, the duration of evoked bursts was markedly reduced, to about 50 ms. In picrotoxin-containing medium at 5 DIV, the first evoked burst was followed by a sequence of bursts of reduced amplitude, separated by more than 200 ms. At 9 DIV, the number of bursts evoked by a single stimulus in picrotoxin was increased, and the interval between successive bursts was shorter than at 5 DIV. This suggests that inhibitory synapses develop between 5-9 DIV and play an important part in modulating the pattern of synchronized bursting.The results suggest that, like dissociated cultures of cortical neurons (Robinson *et al.*, 1993, *J. Neurophysiol.* **70**:1606), cultured organotypic slices have the capacity for propagation of locally-synchronized bursting activity, that the pattern of propagation is determined by the anisotropic structure of the local circuitry of the cortex, and that there is a progressive sharpening in the local synchronization of bursting with development.

706.12

DEVELOPMENT OF CORTICOCOLLICULAR CELLS IN NORMAL AND ANOPHTHALMIC MICE. M. KHACHAB* and L.L. BRUCE, Dept. Biomed. Sci., Creighton University, Omaha, NE 68178.

Anophthalmic corticocollicular (CC) axons show a delayed growth into the superior colliculus (SC) when compared to normal CC axons. This delay may be due to a delayed development of the anophthalmic visual cortex or to the absence of retinocollicular (RC) axons (Khachab and Bruce, Soc. Neurosci. Abstr. 1994). To test these possibilities, the development of anophthalmic CC neurons was compared to that of normal CC neurons. Injections of the fluorescent dye, DiI, were made in the SC of normal (C57BL/6J) and anophthalmic (129SV/CPor^{r}) embryonic and postnatal mice to retrogradely label the CC neurons. In normal mice, labeled cells were first observed at E15. These cells had a short apical dendrite that had not yet extended to the superficial layers. By P0, the apical dendrites extended to the superficial layers and 1 or 2 basal dendrites were present with no collaterals. At P2, the basal dendrites had multiple branches and most apical dendrites were bifurcated. At P4 and P6, the main shaft of the apical dendrites ramified in the superficial layers forming an elaborate terminal tuft and the basal dendritic tree continued to arborize. In contrast, cells in anophthalmic mice were less mature at comparable ages. By P0, only a few dendrites and a fine axon were present. By P6, some apical dendrites had bifurcated and all appeared to extend to the superficial layers with a fine terminal tuft. Basal and apical dendrites had relatively few branches and dendritic collaterals. Thus, a delay in the maturation of the anophthalmic CC neurons appears to be responsible for the delayed growth of the anophthalmic CC axons rather than the absence of the RC axons.

706.13

DENDRITIC DEVELOPMENT OF LAYER 4 STELLATE CELLS IN CAT VISUAL CORTEX. C.J.Pace, S.B.Tieman*, D.G.Tieman, and L.F.Henry. Neurobiology Research Ctr., SUNY at Albany, Albany NY 12222

The visual cortex of the cat develops many of its physiological and anatomical characteristics after birth. However, there are few studies on dendritic development in the visual cortex. We have studied the postnatal development of the dendritic arbors of layer 4 stellate cells. These cells are the primary recipient of visual information from the lateral geniculate nucleus. We intracellularly injected individual neurons with biotinylated Lucifer Yellow within 300 micron fixed cortical sections and processed the sections by the ABC method. Layer 4 stellate cells were entered into a computer and analyzed in 3 dimensions using Sholl concentric rings. In newborn kittens these cells already had most or all of their primary dendrites and fairly extensive dendritic arbors. Branching increased substantially within the first week and only slightly over the next 3 weeks. Dendrites grew in length for at least the first 4 weeks. Additions to dendritic length were proximal as well as distal since the length of first order branches increased. These results demonstrate that dendritic development is coincident with the period of synaptogenesis and mostly precedes that of sensitivity to experience dependent modification.

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706.14

LESION OF CHOLINERGIC BASAL FOREBRAIN NEURONS DOES NOT AFFECT FUNCTIONAL DEVELOPMENT OF THE RAT VISUAL CORTEX. R. Siciliano, N. Berardi, F. Casamenti° and L. Domenici*. Istituto di Neurofisiologia CNR, Pisa 56127 (Italy); ° Dept Pharmacol, Firenze (Italy).

Recently it has been shown that antagonizing cholinergic receptors affects visual cortical plasticity during early postnatal development (Q. Gu and W. Singer, Eur. J. Neurosci. 5:475-485, 1993). Here we tested whether in absence of the cholinergic input from the basal forebrain (BF) the functional properties of visual cortical neurons develop normally. Six rats (Long Evans) were deprived of cholinergic input to the visual cortex at the beginning of the critical period (postnatal day 15) by unilateral stereotaxic injections of quisqualic acid in BF cholinergic nuclei. One month later we performed extracellular recordings under urethane anaesthesia to assess the ocular dominance distribution and orientation selectivity of visual cortical neurons in area 17. At the end of recording sessions rats were sacrificed and the extent of the cholinergic depletion was assessed in terms of the acetylcholinesterase pattern and by measuring the choline acetyltransferase (ChAT) activity at the level of area 17 in both hemispheres. We found that lesion of BF cholinergic nuclei did not substantially affect either the ocular dominance distribution or the orientation selectivity of area 17 neurones.

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706.15

NEURONS IN THE STRIATE CORTEX OF FOUR-WEEK POSTNATAL KITTENS EXHIBIT ADULT-LIKE INHIBITORY PROPERTIES. E.S. Green, G. C. DeAngelis, and R. D. Freeman*. Group in Vision Science. University of California, Berkeley, California 94720-2020.

Excitatory characteristics of neurons in the kitten's visual cortex are thought to be largely mature by four weeks postnatal (Mitchell and Timney, 1984.) However, the status of inhibitory properties at this age is not clear. We have studied specific inhibitory phenomena (end- and side-inhibition and cross-orientation suppression) using single cell recordings in the visual cortex of four-week kittens, and have compared our findings with data from adults.

Responses were recorded extracellularly from neurons in area 17 of paralyzed and anesthetized kittens. To elicit excitation, we used drifting sinusoidal gratings of optimal parameters. To test for end- and side-inhibition we varied the length and width of the excitatory grating. The tuning properties of end- and side-inhibition were studied by varying the orientation and spatial frequency of patches located outside of the excitatory receptive field. To measure cross-orientation suppression the excitatory grating was overlaid with a drifting grating of orthogonal orientation.

Our results show that all three types of inhibition are present in neurons of kittens at four weeks postnatal. As in adults, every cell tested in the kittens exhibited cross-orientation suppression, and maximal cross-orientation suppression could be elicited from a region of approximately the same size as the classical receptive field. Neurons showing end- and side-inhibition to a similar degree, neurons showing only end-inhibition, and neurons showing only side-inhibition were also present in kittens, and occurred in roughly the same proportions as in cats. Finally, end- and side-inhibition were well-tuned for orientation and broadly tuned for spatial frequency, as in adults. These experiments show that intracortical inhibitory mechanisms are quite mature by four weeks postnatal, and suggest that inhibitory and excitatory processes develop in parallel. (EY01175)

706.16

Changing Patterns of BDNF and NT-4/5 Immunoreactivity During Visual System Development. R. I. Cabelli*, S. Tavazoie, and C. J. Shatz, HHMI and MCB, University of California, Berkeley, 94720.

Roles for neurotrophins have been implied in both the selective cell death and activity-dependent competitive remodeling of axons that occur during the maturation of the mammalian visual system. In particular, infusion of exogenous BDNF or NT-4/5 into visual cortex has been shown to prevent the formation of ocular dominance columns. In addition, subplate neurons, which undergo massive cell death during development, have been shown to express trkB, the receptor for BDNF and NT-4/5, and stain with a pan-neurotrophin antibody. Here we have used a polyclonal antibody specific for BDNF, and 2 monoclonal antibodies directed against different epitopes within NT-4/5 to characterize the localization of these neurotrophins in the ferret visual system. During early neonatal development (postnatal days 2-6) first subplate neurons and then cortical layer 5 neurons become strongly immunoreactive for both BDNF and NT-4/5. Staining of subplate neurons subsequently decreases, disappearing between P22 and P31. Neurons in layer 2/3 become immunoreactive by P17 while neurons in layer 4 begin to stain for NT-4/5 but not BDNF by P32, which corresponds to an early stage in ocular dominance column formation. At ensuing ages BDNF immunoreactivity remains low in layer 4 and in the adult BDNF containing neurons are seen predominantly in layers 2/3, 5, and 6; while scattered layer 4 neurons are only lightly stained. In contrast, NT-4/5 immunoreactive neurons are stained with equal intensity within all cortical layers. The localization of BDNF and NT-4/5 suggests that 1) that subplate neurons, which take up injected NT-4/5, may depend on ligands of trkB for their survival during development, and 2) endogenous NT-4/5 or possibly BDNF in layer 4 or other layers could play a role in ocular dominance column formation. Supported by EY02858 (CJS).

706.17

RECIPROCAL LAMINAR LOCALIZATION AND DEVELOPMENTAL REGULATION OF BDNF AND NT-3 mRNA DURING VISUAL CORTEX DEVELOPMENT. E. Lein, A. Hohn* and C.J. Shatz, HHMI and Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

The segregation of thalamocortical afferents into ocular dominance columns (ODCs) that occurs during visual system development in higher mammals is an activity-dependent process in which inputs from the two eyes compete for synaptic space within layer IV of visual cortex. One possible mechanism for this process, which involves the selective growth and retraction of axonal branches, is competition for a trophic substance released by the target neurons. A growing body of evidence implicates the family of neurotrophins in this process and other forms of synaptic plasticity.

A potential role in ODC plasticity for two of the neurotrophins, BDNF and NT-3, was assessed using *in situ* hybridization to localize gene expression in cat visual cortex before, during, and after the critical period for segregation of thalamocortical afferents in layer IV. The expression patterns of these two neurotrophins were found to be reciprocal, both temporally and in terms of cellular localization. BDNF mRNA expression is hardly detectable in visual cortex soon after birth (P7), but increases dramatically after eye opening (P15), where it is first in found in large, presumably pyramidal neurons in deep cortical layers. By the height of the critical period (P28), the pattern is similar to that in the adult, with high levels in large neurons in both deep (V,VI) and superficial (II,III) layers. In contrast, NT-3 mRNA is expressed at high levels in visual cortex prior to the onset of the critical period (P7), mainly in small, presumably stellate neurons in the deep portion of layer IV (IVB). This cortical expression steadily declines and is almost undetectable by the end of the critical period (P45). In neither case was glial neurotrophin expression observed.

This dynamic change in neurotrophin expression during the critical period for thalamocortical segregation suggests a role for these factors in the selective growth and stabilization of eye-specific connections during ODC formation. Supported by NSF predoctoral fellowship (EL) and NIH Grant E402858 (AH).

706.18

DEVELOPMENT AND PATTERN OF OCULAR DOMINANCE COLUMNS IN FERRET VISUAL CORTEX E.S. Ruthazer*, G.E. Baker† and M.P. Stryker. Neuroscience Graduate Program, Dept. of Physiology and Keck Center for Integrative Neuroscience, UCSF, CA 94143, †Dept. of Human Anatomy, Univ. of Oxford, UK

We have examined the developmental time course and spatial pattern of ocular dominance column segregation in the sable ferret visual cortex using transneuronal cortical labeling following injection of either ^{3}H-proline or WGA-HRP into one eye.

In adult ferrets the visual cortex consists of a large contralaterally dominated monocular segment and a binocular segment near the area 17/18 boundary. In addition, and unlike normal cats, in the normal sable ferret a band about 0.5mm wide along the area 17/18 border is completely dominated by inputs serving the contralateral eye. In the binocular segment, ocular dominance columns are distinctly labeled in layer IV and more faintly in layer VI and show a patchy banded appearance, much like columns in the cat. Columns are also present in area 18 but are wider than those in area 17.

In postnatal day (PND) 30 ferrets a largely uniform distribution of label along layer IV was observed in the binocular segment. By PND 37 there was a clearly periodic modulation in the pattern of label in layers IV & VI, resembling that in the PND 21 cat. Over the following six weeks the modulation became progressively sharper in both hemispheres, suggesting increased segregation of the inputs serving the two eyes.

The onset of ocular dominance column segregation occurs about one week earlier in the ferret than the cat (dated from conception) and from the earliest stages of segregation includes a contralaterally dominated zone at the area 17/18 border not seen in normal cats. *Supported by NIH EY027841, NIH T32-EY07120 and a Kleberg Fellowship.*

706.19

'GITTER CELLS' IN DEVELOPING CORTICAL WHITE MATTER ARE INVOLVED IN AXON PHAGOCYTOSIS AND TRANSFORM TO RESTING MICROGLIA M. Schweizer* and C.M. Müller; Max-Planck-Institute for Developmental Biology, 72076 Tübingen, Germany

In early postnatal development thalamo-cortical projections of the mammalian visual system undergo an activity-dependent reorganization giving rise to ocular dominance and orientation maps. The adaptive changes include axon and synapse elimination. A mechanism of such regressive plasticity may reside in the phagocytotic capacity of glial cells. We tested this hypothesis by injecting the lipophilic tracer Di-I into the thalamic lateral geniculate nucleus of kittens aged between 2 and 8 weeks and monitoring transcellular staining due to phagocytosis (*Thanos et al., TINS 17:177, 1994*) after survival times of one to 12 weeks. Fluorescent microscopy revealed Di-I staining at three distinct locations:
i. retrogradely labeled layer VI pyramidal neurons, ii. a band of anterograde label in layer IV, and iii. a distinct population of small cells in the white matter (WM) underlying the visual cortex. Double labeling experiments with the astroglial marker GFAP and the microglial/macrophage marker GSA-I-B4 identified these cells as 'gitter cells'. Transcellular staining in WM was only observed when the dye injection was performed prior to the third postnatal week, coincident with the transient presence of 'gitter cells' up to that age. No transcellular staining of glial cells was observed in the cortical gray matter. The faith of gitter cells was assessed by GSA-I-B4 staining 10 weeks after Di-I tracing performed at an age of two weeks. Cells in the WM, transcellularly stained by the early Di-I injection, now revealed the typical ramified morphology of resting microglial cells. We conclude that 'gitter cells' are involved in the phagocytosis of neuronal structures in early postnatal development and lateron transform to resting microglia. The failure to observe transcellular labeling of glial elements during the period for activity-dependent plasticity challenges a phagocytotic role of glia as a mechanism of regressive plasticity, i.e. axonal pruning. (Supported by the BMFT 0316902)

706.20

MAJOR COMPONENTS OF THE THALAMO-HYPERSTRIATAL SYSTEM ARE ESTABLISHED BY THE TIME OF HATCHING IN CHICKS (*GALLUS GALLUS*) Chi-Cheng Wu* K. Cox, and Harvey J. Karten , Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093

Connections of the thalamo-hyperstriatal system of hatchling chicks were investigated using multiple injections of cholera toxin subunit B (CTb) in the wulst. In the ipsilateral telencephalon, we observed CTb-labeled cells in hippocampus, lateral portion of neostriatum frontale, neostriatum intermedium, dorsolateral portions of neostriatum caudale, posterodorsolateral neostriatum, and other portions of the wulst. We observed CTb-labeled fibers in the paleostriatal complex. CTb-labeled cells were also seen bilaterally in the archistriatal complex. In the diencephalon, CTb-labeled cells were seen in dorsolateralis anterior thalami complex, n. superficialis parvocellularis, and medial and posterior nuclei of the dorsal thalamus. Heavily labeled fibers/terminals were present in the external half of the external laminae of geniculatus lateralis, pars ventralis. Moderate to minor concentrations of labeling were found in n. intercalatus thalami, ventrolateralis thalami, and geniculatus lateralis, pars dorsalis principalis. Evidence of efferent projections of the wulst was observed in the ventral half of optic tectum and many pretectal areas, including n. basal optic root, pretectalis medialis, spiriformis medialis, principalis precommissuralis, and lentiformis mesencephali, pars magnocellularis. In the brainstem, CTb-labeled cells were observed ipsilaterally in n. tegmenti pedunculo-pontinus, pars compacta, substantia grisea centralis, area ventralis, and locus ceruleus, and observed bilaterally in n. tegmenti ventralis and linearis caudalis. The present data demonstrates that major neuronal connections of the thalamo-hyperstriatal system are well established by the time of hatching in chicks.
Supported by NIH NS24560 (HJK).

AXONAL REGENERATION III

707.1

ADULT CENTRAL NERVOUS SYSTEM AXONS DO NOT REGENERATE ACROSS A MICROLESION IN WHICH THE GLIAL FRAMEWORK IS ALIGNED. S.J.A. Davies, P.M. Field and G.Raisman*. The Lee Research Centre, Division of Neurobiology, NIMR, Mill Hill, London NW7 1AA, UK.

Microlesions were made by stereotaxically penetrating the cingulum with a 50μm diameter micropipette. The cut ends of the caudally directed cingulate axons arising from the cholinergic basal forebrain neurons were identified by immuno-histochemistry of p75. From 1 day post-lesion, the proximal parts of the cut axons were expanded into irregular terminal and preterminal varicosities, and emitted a variety of fine sprouts. At no time after the lesion did any of the axons or sprouts advance beyond the line of the lesion. At survival times up to 2 days OX42 staining showed that the lesion site had been infiltrated by macrophages. However by 4 days only ramified microglial cells with thickened processes aligned along the longitudinal tract axis were present within the lesion site and extended as a flare for about 600μm caudally (i.e. in the orthograde direction). This microglial response had largely subsided by 8 days after operation. From 3 days there was an increase in both GFAP and vimentin immunoreactivity of the tract astrocytes at the lesion site. Hypertrophy of the astrocytic processes tended to obscure their original longitudinal orientation. This was reduced to virtually normal levels at 8 days, by which time the arrangement of the tract astrocytes and their processes across the lesion site was similar to that of an undamaged tract.

Thus, although the framework of glial cells and their processes in the cingulum had resumed its normal alignment by 8 days, the cut axons and their sprouts showed no signs of advancing not only during the initial phase of glial response, but also for one week after this response had subsided. These observations indicate that the failure of regeneration of adult axons is not due solely to unavailability of aligned glial pathways.

707.2

AN *IN VITRO* MODEL OF THE DORSAL ROOT ENTRY ZONE.
J. Golding, D. Shewan, M. Berry and J. Cohen. SPON: Brain Research Association. Div. of Anatomy and Cell Biology, UMDS-Guy's Hospital, London, UK.

The failure of lesioned axons from dorsal root ganglion (DRG) sensory neurons to grow into the adult mammalian spinal cord has been attributed to the presence of astrocyte-derived axon-growth inhibitory molecules at the dorsal root entry zone (DREZ). To analyse the cellular and molecular mechanisms involved, we have developed a culture model of the DREZ, using longitudinal cryosections of dorsal adult rat spinal cord, with attached dorsal roots (DR), as a culture substrate for dissociated rat DRG neurons, ranging in age from embryonic (E)13 to adult. After culturing for 1-2 days, sections were triple-stained with antibodies to laminin (to identify DR endoneurium and pia), GFAP (to identify astrocytes at the DREZ) and GAP-43 (to label neurites and their growth cones). The DR supported robust neurite outgrowth from neurons of all ages studied, in contrast to the age-dependent growth we previously described on sciatic nerve cryosections (Bedi, K.S. *et al; Eur. J. Neurosci.* 1992). Furthermore, the longitudinally aligned endoneurial tubes in the DR acted as a conduit for growing neurites, thereby maximizing the number of growth cone encounters with the DREZ. In agreement with previous *in vivo* studies, the majority of neurites growing on the DR failed to cross the DREZ. In contrast, the DREZ posed no barrier to neurites extending from neurons on the spinal cord. Irrespective of neuron age, a small proportion (< 10%) of neurites approaching the DREZ from the DR did cross onto spinal cord tissue and, in the case of neurons isolated from E13-E17 rats, unlike older neurons, subsequent growth on the spinal cord was extensive. The accessibility of this culture model to studies of DRG growth cone interactions with the immature and adult DREZ, from both intact and pre-lesioned rats, should help elucidate the mechanisms underlying the failure of axon regeneration across the DREZ. Supported by Action Research.

707.3

THE CRITICAL PERIOD FOR GROWTH OF DORSAL SPINOCEREBELLAR AXONS THROUGH A LESION OF THEIR SPINAL PATHWAY. STUDIES USING THE NORTH AMERICAN OPOSSUM, DIDELPHIS VIRGINIANA. J.R. Terman*, X.M. Wang, and G.F. Martin, Dept of Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH. 43210.

Opossums are born at fetal stages of development, 12-13 days after conception, making it possible to manipulate them experimentally without intrauterine surgery. We have taken advantage of the opossum's embryology to show that axons of the dorsal spinocerebellar tract (DSCT) are able to grow through a lesion of their spinal pathway from postnatal day (PD) 5-9 (Terman et al, 1994). Such growth does not occur in adult opossums (unpublished observations), but the critical period for it has not been determined. In order to address that issue, opossum pups ranging in age from PD13-68 and still attached to the nipple were anesthetized so that their spinal cord could be hemisected at T8 or T9. They were maintained with their mother for 1-4 months postoperatively before being subjected to bilateral injections of Fast Blue or Fluoro-Gold into the anterior lobe of the cerebellum, the major target of DSCT axons. The intent of the injections was to label any neurons in Clarke's nucleus (CN), caudal and ipsilateral to the lesion, that supported axons which reached the cerebellum. In the opossum, as in other species, DSCT axons originate within CN and project ipsilaterally to the cerebellum (unpublished results). After 7 days survival, the pups were reanesthetized and perfused intracardially with buffered formalin so that their spinal cord could be removed, sectioned transversely, and examined with a fluorescence microscope. In all cases, neurons in CN rostral to the lesion were labeled bilaterally in roughly equal numbers. Labeling in CN caudal to the lesion, however, was limited to the unlesioned side. This implies that by PD13, DSCT axons do not grow through or around a lesion of their spinal pathway to innervate the cerebellum. In contrast, at least some brainstem spinal axons grow through or around a comparable lesion until PD26 (Wang et al, 1994; Martin et al, 1994). Our results suggest that the critical period for growth of DSCT axons through a lesion of their spinal pathway ends considerably earlier than that for comparable growth of brainstem axons (Supported by NS-25095 and 10165).

707.4

ABSENCE OF PROGRESSIVE NECROSIS AND CAVITATION AFTER SPINAL CORD CRUSH INJURY IN MICE, M. Fujiki*, Z. Zhang, L. Guth and O. Steward Dept. of Neurosci., Univ. of Virginia, Charlottesville, VA 22908. Dept. of Biol., College of William and Mary, Williamsburg, VA 23185.

A new approach to CNS regeneration research involves the use of animals carrying particular mutations (natural-occurring or induced). In the course of studies of the effects of mutations on the response to spinal cord injury in mice, we discovered that at least one substrain of mouse (the C57BL/6J substrain) does not exhibit the progressive necrosis and cavitation that characterize the response to spinal cord injury in other species. The spinal cord of adult C57BL/6J mice was crushed at T8 using an extradural approach and animals were allowed to survive for 1, 2, 3, 4 and 8 weeks. The spinal cords were prepared for histological evaluation using the Bodian stain for axons, and the trichrome stain for connective tissue. Reactive changes in astrocytes were evaluated using GFAP immuno-histochemical techniques. The evolution of cellular changes following crush injury in mice differed from what has been described in other species. The injury completely destroyed neural tissue at the crush site. Over time, the area of the crush was essentially completely filled in with non-neural tissue including phagocytic macrophages and connective tissue. Even at the longest intervals (8 weeks post-crush) the two ends of the spinal cord remained connected by an area that was filled with cells and connective tissue with no cystic cavities. Astrocytes were not present within the crush site, but astrocytic processes did form a glial scar at the edge of the crush. At 8 weeks post crush, intact axons could be seen growing amongst the non-neural cells in the crush site. These results indicate that mice have naturally solved what is taken to be one of the key problems facing attempts to repair the injured spinal cord -- the prevention of progressive cavitation and necrosis. Thus, mice will provide a useful model system in which to explore ways to promote axonal regeneration following spinal cord injury. Supported by NIH Grant NS 32280.

707.5

REGENERATION OF THE VESTIBULAR NERVE IN CATS. G. Li*, J. Elidan, A. Newman, I. Lopez, and V. Honrubia. Otolaryngology. Hadassah-University hospital, Jerusalem, Israel; Victor Goodhill Ear Center, UCLA Sch. of Med., Los Angeles, CA. 90024

The purpose of this study was to examine the vestibular nerve regeneration in cats. Under general anesthesia and in aseptic conditions, the cats (n=3) were subjected to a VIIIth nerve (vestibular part) neurotomy on both sides by suboccipital approach. The animals were allowed to survive up to a maximum of 6 months. Short latency vestibular evoked potentials (VsEPs) were recorded in response to angular acceleration impulses. Auditory nerve-brainstem response (ABR) was also used for monitoring the cochlear function. Following the nerve transection, the VsEPs disappeared immediately. At two months post transection time (PTT), the VsEPs reappeared with P_1 and P_2 waves of longer latencies and smaller amplitudes. Four months PTT. P_3-P_4 waves were clearly recognized with P_1-P_2 latencies recovered to normal range. At the 6th month PTT, the amplitudes of the P_1-P_4 waves dramatically increased and the VsEPs had an appearance close to that observed before the transection. On the other hand, ABR showed that the cochlear nerve was partially transected. No ABR recovery was observed until the 6th month PTT. Histological examination indicated that the Scarpa's ganglion showed a full compliment of ganglion cells and the dendrites were identified projecting to the cristae and maculae which were in a normal morphology. In the centrifugal portion of the vestibular nerve and the brainstem, the nerve fibers had lost their orderly pattern. The thicker and thinner fibers intermingled each other. Protargol stained specimens demonstrated that these fibers were long, tortuous and somewhat unusual trajectories comparing to control. However, these fibers finally projected to the neurons in all the vestibular nuclei. Degeneration was observed in the partially transected cochlear nerve. Therefore, the histological results supported the physiological findings (ABR and VsEPs) following the nerve transections. The present study suggests that the vestibular nerve is able to regenerate following the axon transection in cats. (Supported by Oberkotter Foundation, U.S-Israel Bilateral Foundation No.89-00191, and NIDCD grant DC01404)

707.6

INTRACELLULAR DENDRITIC ANALYSIS OF DENTATE GRANULE CELLS FOLLOWING UNILATERAL ENTORHINAL CORTEX LESIONS. K.R. Bulsara*, G.K. Pyapali, A.K. Shetty, and D.A. Turner. Neurosurgery and Neurobiology, Duke Univ. Med. Ctr. and Durham VAMC, Durham, NC, 27710.

Unilateral electrolytic lesions of the entorhinal cortex result in deafferentation of ipsilateral dentate granule cells. Recovery in this deafferentation model has been suggested to be due to terminal proliferation and synapse formation of the crossed temporodentate pathway. However, enhanced electrophysiological activation of granule cells by the crossed temporodentate pathway occurs within a few days following the lesion, preceding anatomical correlates of synapse formation. Thus, early recovery may result from either changes in granule cell dendrites (such as sprouting or atrophy) or synaptic terminals rather than re-innervation. We investigated dendritic alterations in granule cells (6-8 week male F344 rats) *in vitro* at ten days post-lesion by using intracellular neurobiotin staining. Adequately labeled cells were reconstructed on a computerized 3D microscope (Neurolucida).

Normal granule cells show typical unipolar architecture, branches reaching the hippocampal fissure and a total dendritic length of $3.55 \pm 0.84 \times 10^3$ μm (n=10), in agreement with previous data. Preliminary results at ten days following unilateral entorhinal cortex lesions show a total dendritic length of 2.43×10^3 μm. The average width of the entire molecular layer was reduced by 14% in lesioned animals to 189 ± 6.4 μm compared to 220 ± 8.5 μm in normal animals. Morphologically, granule cell dendrites in the outer molecular layer of lesioned animals appeared tortuous as they extended to the hippocampal fissure, possibly due to shrinkage.

These results indicate that loss of the perforant pathway may lead to shrinkage of the molecular layer and possibly secondary dendritic alterations in granule cells. This format of analysis may provide a method to assess both the physiological and anatomical aspects of early functional recovery following the perforant path lesion. Supported by HHMI (KRB), NIA (GKP), NINDS (DAT) and VAMC (DAT).

707.7

STRUCTURAL CHANGES IN ABDUCENS INTERNUCLEAR NEURONS FOLLOWING THE TRANSECTION OF THE MEDIAL LONGITUDINAL FASCICLE IN THE CAT. R.R. de la Cruz*, A.M. Pastor, C. López-García, F.J. Martínez-Guijarro and J.M. Delgado-García. Lab. de Neurociencia, Univ. de Sevilla, 41012-Sevilla and Lab. de Biología Celular, Univ. de Valencia, 4600-Valencia, Spain.

The internuclear neurons of the cat abducens nucleus project selectively on the medial rectus motoneurons of the oculomotor nucleus. Their axons cross midline at the level of the abducens nucleus and course through the medial longitudinal fascicle (MLF) for a length of about 10 mm in the adult cat. We have studied this projection as a model to characterize the response of central nervous system neurons to the injury of their axons. The sectioning of the MLF was performed by a microknife aimed through the cerebellum with an anteriorly directed angle of 45 deg to transect the MLF ~1 mm caudal to the trochlear nucleus. Abducens internuclear neurons observed at 6, 14 and 28 days post-lesion showed marked ultrastructural changes. In contrast to controls, axotomized cells had a disorganized and dilated rough endoplasmic reticulum and a hypertrophic Golgi apparatus. The density of axosomatic synapses was noticeably reduced in the axotomized neurons; instead numerous filamentous or multilayered glial processes appeared covering large areas of the somatic membrane. Postembedding immunocytochemistry of semithin sections against GFAP indicated an increase in immunopositive profiles both in the neuropil and surrounding the somata. Biocytin-labeled axons of abducens internuclear neurons could be followed in parasagittal sections along the MLF caudal to the lesion site. Axons did not penetrate across the scar tissue, but coursed in abnormal trajectories like U-turns and right angles. Axonal terminals formed either big and smooth clubs or sprouted into several short and thin collaterals exhibiting both terminal and *en-passant* bouton-like structures. In spite of the failure of axons to pass through the lesion site, the presence of these bouton-like elements in the MLF caudal to the lesion might represent some form of axonal regeneration in the central nervous system.

707.8

SPECIFICITY OF MOTOR AXON REGENERATION IN "PURE MOTOR" NERVES. T.M. Brushart,* Y.G. Chen. Depts. of Orthopaedic Surg. and Neurology, Johns Hopkins, Balt., MD 21287.

Motor axons regenerating in mixed nerve preferentially reinnervate former motor pathways. However, the influence of regenerating sensory axons on this process in unknown. These experiments examine the specificity of motor axon regeneration after DRG excision. "Pure motor" femoral nerves were prepared in 1 month old rats by excision of the ipsilateral L2, L3 and L4 DRG's. Three groups of 20 experimental nerves were prepared by DRG excision and simultaneous repair of the proximal femoral nerve, enclosing a ½ mm gap within silicon tube. Groups were evaluated at 2, 3 and 8 weeks by double labeling femoral sensory and motor branches with HRP and FG. Labeled motor neurons were counted and scored as to their projection into the motor branch (M), sensory branch (S), or both branches (double labeled, DL). Mean motor neuron counts (N = 20/gp) were: 2 weeks; M-126, S-57, DL-31: 3 weeks; M-194, S-87, DL-33: 8 weeks; M-279, S-76, DL-25. In the 8 week group, a mean of 834 myelinated axons reinnervated the motor branch and a mean of 517 reinnervated the sensory branch. Motor neurons preferentially reinnervated the motor branch at all times. In comparison with previous studies, specificity was greater and occurred more quickly when sensory axons were removed. Motor neurons retained twice as many axon collaterals when projecting to the sensory branch as opposed to the motor.

707.9

WITHDRAWN

707.10

A QUANTITATIVE EM ANALYSIS OF RAT NERVE DEGENERATION AND REGENERATION. J.M. Kerns* and J.Lanciloti. Anatomy Dept., Rush Medical College, Chicago, IL 60612.

The purpose of this study is to quantify the cellular and extracellular characteristics of nerve degeneration/regeneration using volume fraction and cell density analysis of electron micrographs. Random fields (n=10) were selected from 7 dpo injured nerves (n=4) at regions taken *before, at,* and *after* the front of regenerating axons, determined with the vibrating probe. The mean nerve fiber density dropped significantly in the distal degenerating nerve segments when compared to *proximal* normal nerve; the degenerating fibers were still being removed by macrophages. Conversely, the endoneurial space, which is related to tissue edema, showed a significant increase when compared to normal nerve. Volume fraction values were significantly increased in the distal regions for Schwann cells, macrophages, and fibroblasts, while cell density data suggested the fibroblasts did not increase in cell number. These findings are consistent with the tissue swelling and cellular proliferation characteristics of inflammation. As expected, both volume fraction and cell density values of the regenerating axons diminish at regions more distal from the lesion site. This ultrastructural portrait of sprouting axons regenerating through the terrain of degenerating nerve is consistent with current hypotheses. Finally, these comparisons of specific volume fraction and cell density values may improve our interpretation of MRI studies of injured peripheral nerve.
Supported by NIH grant NS19769, BRSG 507RR5477 and the Enelow Fund.

707.11

OCCURRENCE OF EPIDERMAL NERVE ENDINGS IN GLABROUS AND HAIRY SKIN OF THE RAT FOOT AFTER SCIATIC NERVE REGENERATION. N. Stankovic[1,2], O. Johansson[3] and C. Hildebrand[1]*. Dept Cell Biology[1], Dept Plastic Surgery, Hand Surgery & Burns[2], Faculty of Health Sciences, University of Linköping Linköping, Dept Neuroscience[3], Karolinska Institute, Stockholm, Sweden

The occurrence and distribution of intraepidermal nerve endings in hairy and glabrous skin of the rat foot was examined in normal cases and three months after sciatic neurotomy/suture or a crush lesion. Nerve endings were visualized in cryostate sections with antibodies against protein gene product 9.5. Normal glabrous skin exhibited 23.3 endings/mm length. Neurotomy/suture cases had 6.1 endings/mm. In crushed rats the occurrence was normal, but the intraepidermal nerve endings tended to be abnormally short and occurred mainly in the basal layer of the epidermis. In sections from hairy skin countings were not possible. Subjective evaluation indicated that the occurrence of dermal and epidermal axon profiles usually was deficient after neurotomy/sutute and normal after crush. Skin samples from the contralateral side of operated animals showed a normal occurrence and distribution of nerve endings. Cases subjected to neurotomy/suture showed increased numbers of immunoreactive intraepidermal cells and an abnormally thin epidermis. A deficient regeneration of intraepidermal nerve endings may be one factor behind the unsatisfactory restitution of sensory function after neurotomy and suture.

707.12

ANATOMICAL BASIS OF NERVE REGENERATION IN HUMAN SKIN TRANSPLANTS. G.C.Panzica*°, A.Paraninfo°, A. Garzino°, G.Ramieri§, M.Calcagni°°, M. Stella°°, S. Teich-Alasia^. °Dept. Human Anatomy & Physiology, §Dept.Maxillo-Facial Surgery, °°CTO, Dept. Plastic Surgery and Burn Unit, ^FPSRU, Torino (Italy)

In recent years the study of the human skin innervation received new impulses by immunohistochemical techniques. There are, however, only a few studies dealing with functional markers in both the normal and transplanted skin. Previous investigations performed in our laboratory demonstrated the presence of nervous structures, identified by means of structural markers (PGP 9.5, S-100), also in skin transplants. In the present study we analyzed the immunoreactivity for both structural (PGP 9.5) and functional markers of sensitive nerve fibers [calcitonin gene-related peptide (CGRP), substance P (SP)] in biopsies of skin transplants from several areas ranging from 3 months to 8 years. The PGP 9.5 immunoreactive structures are qualitatively distributed in a similar way in normal and transplanted skin, although a marked reduction was observed in the latest. We detected the presence of innervated and non-innervated Merkel cells, intraepidermal fibers, and, in a limited number, that of capsulated receptors. Immunoreactivity for CGRP is present in almost all the structures detected with PGP 9.5 (including some of the Merkel cells, but not the capsulated receptors), although their total number is greatly decreased. Finally in some samples a very limited number of SP-immunoreactive structures (mainly intraepithelial and intradermal fibers) was observed. Our study shows that regenerating nervous structures in human skin may exhibit immunocytochemical markers indicating a potential functional activity. Their number is greatly reduced in comparison with normal skin, but their qualitative distribution seems not altered, suggesting the regeneration involves all the different classes of nerve fibers innervating the skin. Further quantitative studies are required to understand if functional recovery of the transplant and presence of neurochemical markers might be related. *This work was supported by FPSRU*

707.13

Histochemical Analysis of Sensory and Motor Axons and Neuronal Cell Bodies Following Axotomy of the Median-Ulnar and Sciatic Nerves of Adult Rats. **Macias, M.Y. *, Riley, D.A., Lehman, C.T. Department of Cellular Biology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226**

Current studies on peripheral nerve regeneration do not discriminate the regenerating fibers as either alpha motor or sensory axons. Carbonic anhydrase (CA) and cholinesterase (CE) histochemical activities of nerve fibers distinguish myelinated sensory and motor axons, respectively. In our previous studies of the rabbit sciatic nerve, regenerating sensory axon sprouts appeared in the earlier and in greater numbers than motor axons. A rat model was developed to determine whether axonal regeneration onset is dependent upon the distance of the nerve cell body from the axotomy site; and if so, whether neurons are triggered to sprout following axotomy by an early retrograde signal from the injury site. The right median-ulnar and left sciatic nerves of male Sprague-Dawley rats (225-275g) were studied. Motor and sensory cell bodies are at a similar vertebral level for the median-ulnar nerve; in the sciatic nerve, motor cell bodies are further rostral by 4.0 cm. Both nerves were axotomized, and the proximal stumps sampled 10, 30, or 90 days later. Histochemical staining for carbonic anhydrase and cholinesterase was performed on serial cryostat sections, and the ratio of CA/CE positive regenerating fibers was counted. For transport studies, the nerves were crushed, injected at crush site with 5 μl of 1% WGA-HRP, ligated and axotomized distal to crush site. HRP activity was visualized in the dorsal root ganglia and ventral horn of the spinal cords using tetra-methyl-benzidine histochemistry 12, 24, and 48 hrs. after injection. The CA/CE ratio of positive axons in the median-ulnar and sciatic nerves were: 2.5+0.2SD and 1.6+0.1 in control (non-injured); 7.5+2.0 and 68.2+35 at 10 days; 8.0+2.0 and 16.3+1.6 at 30 days; and 1.0+0.1 and 0.7+0.1 at 90 days, respectively. The transport studies showed dorsal root ganglia labelling for both nerves at 12 hrs. Motor cell bodies of the median-ulnar nerve also labelled at 12 hrs., but those of the sciatic nerve were not labelled until 48 hrs. Results show that axon regeneration is directly related to the distance between soma and axotomy site and are consistent with injured neurons being signaled to regenerate by a retrogradely transported factor. (Supported by NAG-522)

707.14

CELLULAR FACTORS INVOLVED IN NEUROMA FORMATION. D.M. Zhang, R.W. Beuerman, S. Zhao, H. Tran, D. Kline, H. Gould*. Depts. of Ophthalmology, Neurosurgery, and Neurology, LSU Medical Center, New Orleans, LA 70112.

Nerve growth factor (NGF) and its low-affinity receptor (NGFR) have recently been detected in the distal segment of the sciatic nerve 6 hours to 14 days after transection. We have investigated cellular localization of NGFR, growth associated protein (GAP-43), basic fibroblast growth factor (bFGF), and FGFR1 at 2 weeks to 12 months in a monkey neuroma model and in human neuromas. In the monkey 2 weeks after transection, tibial nerve proximal segment immunoreactivity for NGFR was found in the perineurium and less intensely in endoneurium. In both monkey and human neuroma, intense staining for NGFR was associated with disintegrating fibers. We also found staining of Schwann sheaths surrounding masses of axons. Immunoreactivity for NGFR was more intense in 12-month monkey specimens and in human neuromas than in 2-week monkey neuroma. Proximal segments of the neuroma showed equal axonal staining for GAP-43, whereas very few thin disorganized fibers were stained with GAP-43, and the number of GAP-43-stained fibers decreased with time of neuroma development. Western blot of 3-month monkey neuroma showed a decrease in bFGF and FGFR1, compared with the proximal segments. In conclusion, the increase in NGFR, together with the decrease in GAP-43, bFGF, and FGFR1, may be related to the formation of the neuroma. However, the role of NGFR in the development of the axon mass of the neuroma is not clear. Supported in part by DAMD17-93-V-3013.

707.15

Long-term Changes of Motor Unit Organization after Peripheral Nerve Repair

A.Mautes, M.Lehnert, Ch. Braun and A.C. Nacimiento*, Neurosurgical Research Laboratory and Department of Trauma Surgery, Saarland University, Medical School, 66421 Homburg/ Saar

Previous work from our laboratory showed that end-to-end or graft repair of the transected peroneal nerve alters histochemical profile of reinnervated extensor digitorum longus (EDL) muscle in the rat by inducing fiber type conversion leading to an additional fiber type (SDH-INT) with reactivity between those of type IIA and IIB fibers. This type is not present in the normal EDL of young adult rats. An early, bilateral increase in number of SDH-INT 3 months after repair was followed by a decrease after 6 months, indicating partial recovery of motor unit organization pattern. To analyse degree and scope of this reversibility we extended our investigation to 15 months postoperative. After computer assisted quantification of histochemical typing, the values obtained were compared with those of animals with intact innervation of the same age. **Results:** SDH-INT number increased significantly through conversion of IIA fiber type on the operated, but not on the contralateral side after end-to-end repair. No differences were observed after grafting. In intact muscle SDH-INT fiber type was also present, but to a lesser amount. **Conclusion:** Postrepair alteration of motor unit organization was not reversed with time, suggesting a reduction of motor unit plasticity enhanced by an interaction between reinnervation and concomitant age related changes of fiber type composition.

707.16

TYROSINE HYDROXYLASE-IMMUNOREACTIVITY IS EXPRESSED IN DORSAL ROOT GANGLIA OF CHICK EMBRYOS AFTER SPINAL CORD TRANSECTION. P.A. Gurulé, A.Y. Gonzales, K.D. Kemp and J.A. Wallace*. Dept. of Anatomy, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

Catecholaminergic phenotype traits, such as the expression of tyrosine hydroxylase (TH), are normally not associated with cells of the dorsal root ganglia (DRG). However, cells derived from embryonic quail DRG synthesize TH as well as catecholamines when grown in culture, although these properties are not found in normal developing embryos (Xue et al.,`85). In contrast, small numbers of TH-immunoreactive (TH-IR) cells are present in many DRG in chick embryos, and we report here a striking change in the number of these cells in animals that have undergone spinal cord transection. Animals underwent complete mid-thoracic spinal cord transection either on E12 or E14 according to the methods of Hasan et al.`93 (all animals were prepared in the laboratory of Dr. J. Steeves, Univ. Of British Columbia), and were later sacrificed on E20-21. Analysis of embryos transected on E14 revealed an increase in TH-IR cells within DRG throughout the thoracic cord, although the increase was most prominent at the level of initial cord lesion with up to 25-30 TH-IR cells observed per 10 μm section of DRG. The increase in number of TH-IR cells decreased markedly in both cervical and lumbar cord segments. Control unlesioned animals at E21 demonstrated at most 2-3 immunostained cells per section of DRG at any cord level. In embryos lesioned on E14 the TH-IR cells were dispersed throughout the DRG and appeared as primary sensory neurons ranging from 20-30 μm in diameter; some with a single thick immunostained axon. In contrast, no comparable changes were discerned in embryos transected on E12. It is possible that the expression of TH by chick embryonic DRG neurons results from injury to central projections of the cells or appears as a response to synaptic rearrangements and exposure to various trophic factors in the spinal cord during the recovery period. Supported by DHHS-GM08222 and RR08139.

707.17

FORMATION OF SYNAPTIC CONNECTIONS IN THE PTEROPOD MOLLUSC *CLIONE LIMACINA*: REGENERATION AND CELL CULTURE STUDIES. Yu. Panchin, L. Popova*, P. Zelenin, R. Sadreev. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119899, Moscow, Russia.

The neuronal network controlling rhythmic wing movements in the pteropod mollusc *Clione limacina* is located in a pair of pedal ganglia interconnected by commissure (C). Most neurones comprising the network, as well as their connections have been identified. The motoneurons project to the wing muscles via ipsilateral wing nerve (N). The interneurons provide coordination between the left and the right wings by means of their axons in C. It was shown that, in the otherwise intact animals, the original connections of the moto- and interneurons regenerated after crushing their axons. A new technique to study this regeneration *in vitro* was used in the present investigations. The isolated pedal ganglia were embedded in agarose gel and cultured in different positions to each other: NN - the stump of the wing nerve of the right ganglion contacted the stump of the left wing nerve; NC - the stump of the wing nerve of the ganglion contacted the stump of the commissure of the other ganglion; CC - the stump of the commissure of two ganglia contacted each other. Interneurons were able to outgrow only through the CC contact. In this case, they restored correct connections with their original targets. In two other cases the interneurons sprouted in their own ganglia only. The motoneurons were able to cross all three types of contact. They formed chemical connections which were not present in the intact ganglia, the connections resembled the neuromuscular acetylcholinergic synapses formed by the motoneurons on the muscle fibres. The abnormal chemical connections were also found in the pairs of cultured isolated identified motoneurons. (Supported by ISF grant MJU000 and KVA grant for Swed.-Russ. sci. coop.).

STAINING, TRACING, AND IMAGING TECHNIQUES III

708.1

DIRECT AND INDIRECT DETECTION OF FLUORESCEIN LABELLED NUCLEIC ACID PROBES FOR IN SITU HYBRIDIZATION. I. Durrant* and S. Brunning. Amersham Laboratories, Amersham, Buckinghamshire, HP7 9LL, UK.

In situ hybridization is a powerful technique for the study of mRNA, genomic sequences and viral infection within the structure of cells, tissues and chromosomes. A non-radioactive system has been developed which uses fluorescein as a label for DNA, RNA and oligonucleotide probes. The efficiency of the labelling reaction can be monitored in a rapid, semi-quantitative assay based on the fluorescent properties of the label. The system uses a hybridization buffer which is formulated to enhance specific signal and reduce background. Various detection procedures have been utilised to reveal the fluorescein labelled hybrids. Comparison has been made in terms of resolution, sensitivity and speed for direct and indirect detection of the fluorescein label. The direct method utilises fluorescence microscopy whilst the indirect methods utilise anti - fluorescein antibody conjugated to either alkaline phosphatase, HRP or gold and detected using appropriate substrate systems. These include NBT / BCIP, cobalt enhanced DAB, luminol based chemiluminescence or silver enhancement. Model systems used in this work including POMC detection in rat pituitary sections.

708.2

A RIBONUCLEASE-RESISTANT AND REPRODUCIBLE METHOD OF IN SITU HYBRIDIZATION HISTOCHEMISTRY IN RAT BRAIN TISSUE. M. E. Wolf*, H. Y. Chen and W. X. Lu. Dept. of Neuroscience, Finch University of Health Sciences/ The Chicago Medical School, North Chicago, IL 60064

Two major problems limiting neurobiological applications of in situ hybridization are: 1) contamination by ribonuclease (RNase), which is difficult to avoid and therefore makes the method difficult to establish for many laboratories, and 2) lack of reproducibility, which makes the method inadequate for detecting and quantifying changes in mRNA levels. Based on a previously described method for free-floating brain tissue (Brain Res. 578: 155, 1992), we have developed a modified method of in situ hybridization that addresses these problems. Briefly, rat brains are perfused with saline containing 0.02% diethyl pyrocarbonate, a RNase inhibitor, followed by fixative solution (4% paraformaldehyde). Brains are cut frozen (40 μm) and sections stored free-floating in an ethylene glycol-based cryoprotectant solution at -20°C. Brain sections are then rinsed in 50% formamide and 4 x SSC, and hybridized with ^{35}S-labeled oligonucleotide probes at 37°C overnight. Thus, RNase resistance is afforded by the inclusion of RNase inhibitors during steps in which mRNA is vulnerable to RNase digestion, alleviating the need to maintain RNase-free conditions during experiments. These changes result in higher levels of specific hybridization, while maintaining low background. In addition, a high level of reproducibility is obtained, both for sections obtained from the same animal and for corresponding sections obtained from different animals. This method has been characterized for preproenkephalin and glutamate receptor GluR 1-4 mRNAs. Suported by USPHS Grant DA 07735.

708.3

PERIAXOPLASMIC PLAQUES ARE NOVEL RNA-ASSOCIATED STRUCTURES LOCALIZED SUBJACENT TO THE AXOLEMMA OF THE MAUTHNER CELL AXON. E. Koenig.* Dept. of Physiology, Univ. at Buffalo, Buffalo, NY 14214.

Recently, ultrastructural analysis of unstained, ultrathin sections of isolated M-cell axons, using electron spectroscopic imaging (ESI) of RNA phosphorous, revealed random clusters of 25 nm signals typical of ribosomes, which appeared only above the phosphorous absorption edge. The clusters were distributed intermittently in the cortical zone and were not present in RNase digested axoplasm (Koenig & Martin *Soc. Neurosci. Abstr.* Vol 20, Part 2, p. 1332, 1994). Surface inspection of isolated M-cell axoplasm after staining with YOYO-1, a selective, nucleic acid fluorescent dye, reveals periaxoplasmic plaque-like bodies, which vary in size, shape, intrinsic organization, and fluorescence intensity. Structural correlates are often visible in phase-contrast and DIC microscopies. Associated with regional domains of some plaques are discrete fluorescent puncta, which are distributed within a volume extending into the ectoplasm. All YOYO-1 fluorecent structures are sensitive to RNase. Confocal microscopy after double labeling of isolated axoplasm with rhodamine-phalloidin and YOYO-1 indicates that the plaques can be superficial to and/or integrated within the cortical actin layer. Periaxoplasmic plaques, which are structurally labile unless stabilized in isolated native axoplasm, may be ribosome-associated domains.

708.4

DIFFERENT RATES OF PROLIFERATION OF HSV STRAINS MAY CONTRIBUTE TO SPECIFIC PATTERNS OF VIRAL SPREAD IN THE CNS. J. H. LaVail,*, A. L. Rothman, S. M. O'Rourke, and K. S. Topp. Dept. of Anatomy and Neuroscience Program, UCSF, San Francisco, CA 94143.

Different viral strains have been shown to correlate with patterns of intercellular transport of Herpes simplex virus (Type 1) (HSV) within the CNS. McIntyre-B strain undergoes retrograde transneuronal transport, i. e., from cell bodies to nearby axons; H129 strain undergoes anterograde transneuronal transport, i. e., from axons to nearby cell bodies. Mechanisms that might account for the different viral behaviors include preferential uptake by axons or cell bodies/dendrites; a defect in anterograde or retrograde axonal transport; and different rates of viral proliferation or release. Our goal was to determine whether the behaviors could be correlated with the abilities of the viral strains to undergo anterograde or retrograde transport or with preferential infection of domains of the plasma membrane. The corneas of BALB/c mice were inoculated with equivalent titers of McIntyre-B or H129 HSV. After 3-5 days, the mice were killed, and the trigeminal ganglia (TG) and brainstem were examined immunocytochemically for the presence of HSV antigens. Both strains were transported in the retrograde direction to TG cells and labeled equivalent numbers of TG neurons. However, the McIntyre-B infected TG displayed fewer lymphocytes or HSV-positive axons or glial cells than the H129 infected TG. Although both strains were capable of anterograde transport of virus, viral infection in the n. of the spinal tract of V in the McIntyre-B infected animals was delayed by one day. We also studied the apical and basolateral plasma membrane domains of MDCK II cells as models for polarized uptake by neuronal axonal and dendritic domains. Half of the cultures were treated with EGTA to disrupt tight junctions between cells and expose the basolateral cell surface. Equal titers of virus were applied to the cultures, and the number of immunopositive foci per pfu inoculated was measured. The strains were identical in their preferential infection of the basolateral surface of the MDCK cells. However, the H129 strain of HSV yielded 2.3-fold the number of foci per pfu as as did the McIntyre-B strain virus. The increased proliferation or viral release of H129 may lead to a more robust anterograde transport of virus following viral inoculation in the CNS.

708.5

ADENOVIRUS TRANSFER OF GREEN FLUORESCENT PROTEIN FOR LABELING PRIMARY MAMMALIAN NEURAL CELLS. K. Moriyoshi[1], L.J.Richards[2], C. Akazawa[2], S. Nakanishi[1], D.D.M. O'Leary[2]*, Inst. for Immunology, Kyoto Univ., Kyoto, Japan[1]& The Salk Inst., La Jolla, CA[2].

The jellyfish protein, green fluorescent protein (GFP), is a bioluminescent protein shown to be effective at labeling touch receptor neurons in transformed C.elegans (Chalfie et al.'94). Due to its small coding region (0.7 Kb), GFP has the potential to be used to tag other gene products (Marshall et al.'95), or as a marker for neural cells in living preparations since it requires no fixation or substrate to be visualized with a fluorescent microscope. GFP has effectively labeled neural cells in worms (Chalfie et al.'94), Xenopus (Wu et al.'95) and zebrafish (Tannahill et al.'95), but not in mammals. We have been modifying the GFP gene and testing the efficacy of different promoters to increase the fluorescence labeling of mammalian neural cells. We placed GFP under the control of the CAG promoter (Niwa et al.'91) and found that in COS cells GFP labeling was bright, although concentrated in the nucleus; but in primary neural cell cultures labeling was dim. To improve the fluorescence intensity we repeated the GFP mutations created by Helm, Cubitt & Tsien ('95). These mutations, in addition to the CAG promoter, produced strong fluorescence. To modify GFP localization, we have also made fusion constructs of the GFP with the membrane associated region of other proteins, and a tandem GFP repeat construct. The fusion constructs produced a strongly fluorescent protein localized to the cell membrane of primary neuronal cultures. By coupling the modified GFP-CAG constructs with the replication-incompetent adenovirus gene transfer system, we are assessing the efficiency of this system for labeling neural cells in vivo.

708.6

A NEURAL CELL-TYPE SPECIFIC GENE EXPRESSION SYSTEM USING RECOMBINANT ADENOVIRUS VECTORS. M. Hashimoto[1], J. Aruga[1], Y. Kanegae[2], I. Saito[2], S. Yoshida[3], Y. Hosoya[4], H. Yaginuma*[4] and K. Mikoshiba[1,2] [1]Mol.Neurobiol. Lab., RIKEN, Tsukuba, Ibaraki 305, Japan, [2]Inst. of Med. Sci., Univ. of Tokyo, [3]Cell and Information Group, RIKEN, [4]Univ. of Tsukuba.

We demonstrated a neural cell-type specific gene expression using adenovirus vector, which is useful for delivering foreign genes into quiescent neural cells. We produced eight recombinant replication-defective adenoviruses carrying the *LacZ* reporter gene driven by various promoters, including those of L7/PCP2 gene (highly restricted expression in cerebellar Purkinje cells), and the myelin basic protein (in oligodendrocytes) gene. We demonstrated *in vitro* and *in vivo* promoter-driven, neural cell-type specific gene expression by recombinant adenoviruses. Primary culture of mouse cerebellum were infected with these adenoviruses. When we can observe *LacZ* gene expression only in Purkinje cells with L7-*LacZ* vector and in oligodendrocytes with MBP-*LacZ* vector. We also introduced these adenovirus vectors into the organotypic slice culture of mouse cerebellum and *in vivo* injection into rat cerebellum. These cell type-specific adenovirus vectors make it possible to study the molecular mechanism underlying, development and functional organization of the nervous system. Thus, adenovirus vectors are useful in cell-type specific therapeutic uses and studies requiring neural cell-type specific gene expression.

708.7

CATIONIC LIPOSOME-MEDIATED EXPRESSION OF BACTERIAL BETA-GALACTOSIDASE (b-gal) IN CULTURED SPINAL TISSUE. H.M.E. Azzazy[1]*, R.C. Benjamin[2], and G.W. Gross[2], (1) Clinical Laboratories, Univ. of Maryland Medical System, Baltimore MD 21201, and (2) Department of Biological Sciences, Univ. of North Texas, Denton TX 76203.

Control expression vectors pCMV-b-gal (Stratagene) and pSV40-b-gal (Promega) were complexed with rhodamine-labeled cationic liposomes (made of DDAB or DOPE and DOTAP, 1:1 mole ratio) and applied to 3-8 week cultures grown from dissociated embryonic (E-14) murine spinal tissue. Cells were incubated with DNA/liposome complexes (2:1 mole charge ratio) for 4 h after which the medium was changed and cells were post-incubated for 48 hr with no resulting overt cytotoxicity. Histochemical staining of these cultures for b-gal revealed strong staining in some glia cells of the carpet. Control staining of non-transfected cells or cells treated only with plasmid DNA was negative for b-gal. So far we have not seen any neurons stained for b-gal. Mechanisms of molecular delivery could be (i) endocytosis, as demonstrated by the localization of intact liposomes inside cells with electron microscopy, and (ii) fusion, because of the strong, uniform fluorescence seen in all neurons (and some glia) treated with rhodamine-labeled liposomes. In light of strong rhodamine staining of neurons and weaker staining of glia, we anticipate that most cells in these cultures received the vectors but that only a few glia were able to express b-gal. We conclude that cationic liposomes hold promise as a delivery system for transformation of mature mammalian neurons.

708.8

FURTHER CHARACTERIZATION OF A NOVEL NEURONAL MARKER FOR CELL LINEAGE STUDIES. I.A. Coleman*, A. Sonshine, and K.A. Kelley. Fishberg Res. Ctr. for Neurobio. The Mount Sinai Sch. of Med., New York, NY 10029.

Thy-1 is a cell surface protein of the immunoglobulin superfamily, expressed on all neurons except those of olfactory epithelial origin. We have attempted to take advantage of the widespread neuronal expression of this gene by using it to produce a novel marker for cell lineage studies in the developing cerebral cortex. Our initial strategy was to use the Thy-1 promoter and regulatory regions to drive the expression of a bacterial marker protein. Due to the complexity and apparent positional dependance of the Thy-1 enhancer elements, we were only moderately successful (Soc. Neuro. Abst., 19: 1260, 1993). Our next approach was to make a minor modification to the Thy-1 gene; the signal sequence was replaced with a unique 8 amino acid sequence ("FLAG" epitope; IBI, New Haven, Conn.). In the resulting cytoplasmic epitope-tagged Thy-1 (CET) transgenic mice, CET transcript was expressed only in tissues that normally express Thy-1 and CET protein was localized to the cytoplasm (Soc. Neuro. Abst., 20: 57, 1994). We now report the production of homozygous mice from one transgenic line. Quantitation of mRNA levels by protection assay suggests that the level of CET transgene mRNA in homozygous mice is about 12 times the level of endogenous Thy-1 mRNA. Preliminary immunocytochemistry in homozygotes reveals widespread expression of the transgene in the cortex. In addition, northern analysis indicates the developmental expression of CET mRNA parallels the expression of Thy-1 mRNA, suggesting that the transgene is regulated in the same manner as the endogenous gene. We are now in the process of identifying, by in situ hybridization, the cell types that express the CET transcript and are optimistic that we will be able to begin our cell lineage studies soon.

708.9

IMAGE CYTOMETRY OF ß-GALACTOSIDASE REPORTER GENE EXPRESSION FUSED TO GAP-43 GENE IN RAT PC-12 CELLS. Y. Liu* and L.E. De Bault. Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

The cDNA encoding the N-terminal 20 amino acid sequence of the neural specific protein GAP-43 (neuromodulin) was fused with the ß-galactosidase (ß-Gal) gene. The fusion gene was subcloned into pcDNA I-neo (Invitrogen) at Hind-III and Not-I sites, under the control of a human cytomegalovirus promoter. The plasmid construct was transfected into a PC-12 (rat pheochromocytoma) cell line which gave a stable but heterogenous population of transfected cells (containing positive cells with a single or multiple copies of the plasmid and negative untransfected cells). Enzymecytochemistry of the ß-Gal was performed using 5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside as the substrate resulting in a blue stain that was measured at 620nm in a DISCOVERY™ *Flu· Or· bance* Image Cytometry System. The positive cells showed a range of staining intensities from a basal level assumed to represent the expression of a single copy of the plasmid to values 4 to 5 times this basal level. In a few cells the expression of the ß-Gal exceeded 10 times the basal level. Further work on a suitable external standard is in progress. When properly controlled this enzymecytochemical procedure combined with quantitative Image Cytometry can be used to monitor gene expression at the individual cell level. (Supported in part by NIH grant NS-18775 and an OCAST to L.E.D. and NSF grant IBN-9119975 to Y.L.)

708.10

HINTS OF CELLULAR RESOLUTION USING RETROGRADE VOLTAGE-SENSITIVE DYE STAINING IN THE EMBRYONIC CHICK SPINAL CORD. Y. Tsau, L.B. Cohen* and C. Hickie. Department of Physiology, Yale University School of Medicine, New Haven, CT 06520

Wenner et al and Tsau et al (SFN, 1994) showed that hydrophobic, positively-charged styryl dyes would retrogradely label motoneurons in the embryonic chick spinal cord after injection into ventral roots. However, the activity-dependent signals on neighboring detectors of the photodiode array were always similar; furthermore, the time course of the signals was relatively smooth. Thus, it seemed that each pixel was detecting the averaged activity from a substantial population of motoneurons. After making several changes in experimental procedure we have detected more localized spike-like signals. First, we used a brighter light source (250 vs 150 watt xenon arc bulb) and an objective with larger magnification and higher numerical aperture (63x 1.25 NA) which improved the signal-to-noise ratio. Second, we used older animals (E14-E16) with the idea that the motoneuron activity might be less synchronous. Third, we used near-threshold stimuli to the rostral cord in an attempt to spread out the timing of motoneuron responses. With these changes we have detected spike-like signals that were localized both spatially and temporally over the photodiode array. Thus, we may be close to being able to detect the activity of individual motoneurons in the embryonic chick spinal cord. Supported by NIH grants NS08437 and NS07102.

708.11

THE EFFECT OF NEUROBIOTIN ON MEMBRANE PROPERTIES AND MORPHOLOGY OF INTRACELLULARLY LABELED NEURONS XL X.Z*. AND XU, Z.C. Dept. of Neurology, University of Tennessee, Memphis. Memphis TN 38163

Neurobiotin has been widely used for intracellular staining. However, little is known about the electrophysiological effects of neurobiotin on recorded neurons and how long it remains within the cell after labeling. Using spiny neurons in rat neostriatum, the present study aims to address these questions with *in vivo* intracellular recording technique.

Male Wistar rats were anesthesized with 1-2% halothane and fixed on a stereotaxic frame. Intracellular recording was performed using electrodes filled with 2M K acetate with or without 3% neurobiotin. Spontaneous activities and membrane properties were compared before and after injecting depolarizing current pulses for 15 min at different amplitude (0.5-1.5nA). The animals were sacrificed at different intervals after recording for histological precesses.

Using electrodes filled with 3% neurobiotin in 2 M potassium acetate, no significant changes in membrane properties were observed if the iontophoresis current was 0.5 nA. If the current was greater than 1 nA, the spike height was decreased and the spike width was increased. Similar changes were observed if the electrode was maintained in the cell for more than 30 min without current injection. With 2 M potassium acetate electrodes, no significant changes were observed after 1nA current injection suggesting that the changes were caused by the neurobiotin. After recording the animals survived up to 48 hours before histological processing. The recovered rate of labeled neurons decreased with longer survival period. The recovered neurons were strongly stained 12 h after experiment and faded significantly at 48 h. No degeneration sign was observed in all labeled neurons. The present study indicates that neurobiotin has no significant effect on membrane properties except the spike width and spike height of the recorded neurons. The neurons can be recovered up to 48 hours after intracellularly stained with neurobiotin.

Supported by grants from NIH NS33103 and AHA 9400813

708.12

TRANSSYNAPTIC TRANSPORT OF INTRACELLULARLY INJECTED BIOTINAMIDE (NEUROBIOTIN) IN PRIMARY AFFERENT AXONS. D. Dessem* and P. Luo. Univ. of Maryland Dental School, Baltimore, MD 21201.

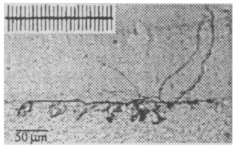
Rats (340-380g) were anesthetized with pentobarbital (40mg/kg IP) and microelectrodes filled with 2% biotinamide (Neurobiotin, Vector) were advanced into the tract of the mesencephalic nucleus of the trigeminal nerve (Vme). Jaw-muscle spindle afferent axons were then identified by their increased firing during stretching of the jaw-elevator muscles and injected with biotinamide (DC 4-8nA, 2-10 min). These animals were then maintained under anesthesia for 2-6 hours, sacrificed and the brainstem was immunohistochemically processed. Biotinamide-filled axon collaterals and terminals were readily visible in the trigeminal motor nucleus, trigeminal sensory nuclei and adjacent reticular formation. In addition, 2 to 5 neurons per animal (36 total in 8 rats) were observed with a homogeneous gray reaction product distributed throughout their somata, proximal and secondary dendrites. These small (8-20μm, n=26) to medium-sized (<30μm, n=10) neurons were closely apposed by numerous (up to 20) biotinamide-stained, spindle afferent boutons. Most of these neurons (n=22) were located in the spinal trigeminal subnucleus interpolaris (Vi) 2.5 - 4.5mm caudal to the intra-axonal injection site. Electron microscopical analysis in 2 rats demonstrated that the transport of biotinamide occurred predominantly through asymmetric, axo-dendritic synapses between biotinamide-filled axon terminals and Vi neuronal dendrites. These synapses typically had a wide synaptic cleft (15-25 nm) and long concavo-convex or sinuous active zone (300-750 nm). Transsynaptic transport of biotinamide through axo-axonic synapses was only encountered occasionally. Recent *in vitro* studies have reported that biotinamide permeates through dendritic gap junctions. By contrast, we found no evidence of biotinamide traversing the gap junctions which exist between Vme neuronal somata. These results demonstrate that biotinamide can occasionally be transsynaptically transported; further information is needed to explain the seemingly sporadic nature of this transport. Supported by NIH DE10132.

708.13

SINGLE-CELL LABELING BY JUXTACELLULAR APPLICATION OF ANTEROGRADE TRACERS. D. Pinault* *Neurobiologie, Université Laval, HEJ, 1401, 18ème rue, Québec, G1J 1Z4, Canada.*

We describe a novel method with unique advantages for the Golgi-like labeling of single neurons identified and recorded extracellularly. This procedure involves the use of fine glass micro-pipettes (tip diameter about 1 µm), which contain biocytin, Neurobiotin or biotin dextran dissolved in a salt solution (1.5% in 0.5 M NaCl or K-acetate), for the simultaneous juxtacellular recording and tracer iontophoresis. Low intensity positive current pulses (< 5 nA, 200 ms on/200 ms off, 5 to 30 mn), injected through the bridge circuit, modulate cell firing such as to keep it alive. Survival periods of 3-24 h (for biocytin or Neurobiotin) and of up to 4 days (for biotin dextran) yielded the best dendritic and axonal stainings. Rat thalamocortical cells, for instance, which were juxtacellularly labeled, could be traced to their most distal axonal processes in the cortex. Electrophysiological and histochemical data show that neuronal filling occurs during a cellular micro-puncture, electrically-induced at a somatic or dendritic membrane patch. Control experiments consisting in the selective killing of previously injected cells provide convincing evidence it is the stained unit that was recorded extracellularly and "tickled" by the juxtacellular iontophoretic pulses. This single-cell staining method has been used to label several types of rat brain neurons, including interneurons. Its success rate (85-100 %) far exceeds that obtained by direct intracellular injections of tracers as shown by the labeling of a large sample of 98 individual cells (from 113 attempts) in the thalamic reticular nucleus of 32 rats.

Provide care is taken to avoid the possible drawbacks and pitfalls that are illustrated and discussed, this novel juxtacellular staining method represents an ideal directed Golgi-like labeling tool for studying relationships between the structure and function of individual central nervous system neurons. *Supported by a grant from MRC of Canada to M. Deschênes.*



708.14

A NOVEL NEURAL TRACER USING THE PIG PARAMYXOVIRUS OF THE BLUE EYE DISEASE. M.A. Ramírez-Herrera, M.L., Mendoza-Magaña and S.H. Dueñas*.CUCS, Dept. of Physiology. Universidad de Guadalajara, Guadalajara, Jal. México. 44340.

This study analyzed weather the pig paramyxovirus of the blue eye disease (PPBED) was retrogradely transported from peripheral nerves to the CNS. In five suckling pigs (3-8 days old) and four cats (300-2500 g) a dose of 1 ml of paramyxovirus with 256 hemagglutinating units was injected in left medial gastrocnemius (MG) muscle or intradermal in the skin of the area innervated by the left sural (SU) nerve, or, it was also inoculated *per nasum*. After 11 - 40 days of injection or 5 -12 days of inoculation the animals were anesthetized with sodium pentobarbital (30 mg/kg) and fixed by intracardial perfusion with paraformaldehyde (6%) and glutaraldehyde (3%). Several distal to proximal segments (5 mm) of left and right (control) GM, SU, sciatic (SC) and nasal (NA) nerves as well as proximal and distal segments of L_5- and L_6 ventral and dorsal roots were cut and processed for paraffin embedding. Transversal sections of spinal cord, brain cortex, hippocampus and cerebellum were also processed for embedding. The presence of the virus was detected by an immunohistochemical method using mouse policlonal antibodies against the whole inactivated virus, which was revealed with rabbit anti-mouse IgG labeled with peroxidase. In pigs, the virus was detected in distal and proximal MG, SU, SC and NA nerves as well as in ventral and dorsal roots. It was not revealed in the control contralateral side. It also appeared in motoneurons of the infected side. In Purkinge and pyramidal neurons of hippocampus and brain cortex only appeared when, it was inoculated *per nasum*. In cats, the virus was only detected in distal portions of the peripheral nerves. It seems possible that PPBED penetrates to the CNS by the neural retrograde transport occurring in peripheral nerves.

708.15

COMPARISON OF THE UPTAKE AND TRANSPORT OF SOME COMMONLY USED RETROGRADE TRACING SUBSTANCES. J. Li* and P.N. Izzo. Dept. of Physiology, Royal Free Hospital School of Medicine, Rowland Hill St, London, NW3 2PF, U.K.

A variety of retrograde tracers have been used over the past two decades to study the projections of central and peripheral neurones. Although many of these substances are well established some of the more recent additions have not been critically examined. The purpose of the present study was to assess the properties of a number of classical and contemporary retrograde tracers and to compare their sensitivity.

Experiments were performed on anaesthetized male Sprague-Dawley rats (250-350 g). Individual tracers (HRP, cholera-toxin (CT) conjugated to HRP, fluorescent latex microspheres (30nm), WGA conjugated to colloidal gold particles (1 and 5nm) and Fluoro-Gold) were pressure injected (10-15 µl) into the tongue or hypoglossal nerve. After a suitable survival period (2-7 days) rats were perfused fixed and serial coronal sections (50 µm) of the medulla (at the level of hypoglossal nuclei) were cut on a vibrating microtome. The retrogradely transported substances were then detected and visualized using the appropriate method. Following tongue injections extensive labelling of hypoglossal motoneurones was observed with CT-HRP and Fluoro-Gold and to a lesser extent with HRP. However, following the injection of either WGA-gold or latex microspheres very little retrograde labelling was observed. In contrast, injection of all the tracers into the hypoglossal nerve resulted in labelling of hypoglossal motoneurones.

This study demonstrates that some of the more recently reported tracers employed in neuronal retrograde tracing studies are indeed retrogradely transported but are not readily taken up by axon terminals. Thus, previously reported retrograde transport achieved with these tracers must be attributed primarily to uptake and transport by damaged fibres at the site of injection. The results from this study suggest that the most specific and sensitive retrograde tracers are cholera-toxin and Fluoro-Gold, both of which are taken up by axon terminals and produce more numerous and extensive labelling of neurones.

709.1

ANTIGEN RETRIEVAL FOR PARAFFIN SECTION IMMUNOCYTOCHEMISTRY WITH ANTIBODIES COMMONLY USED IN STUDIES OF DEGENERATIVE DISEASES. J.C. Hedreen* and L.A. Mucci. Depts. of Psychiatry and Pathology, New England Medical Center and Tufts University School of Medicine, Boston, MA 02111

Paraffin section immunocytochemistry using human brain tissue obtained at autopsy and fixed for several weeks has presented difficulties with sensitivity. Recent methods of pretreatment using wet heat ("antigen retrieval") allow more reliable demonstration of many antigens at higher antibody dilution. The most convenient and effective methods in our hands include 10 min. autoclave or 20-30 min. at 90-95°C. (heating bath) in 0.01M citrate pH 6.0. Background staining is reduced by these pretreatments. A streptavidin-peroxidase kit (Biogenex) and DAB or DAB plus nickel as chromogen is used. With these methods, the following dilutions of commonly used antibodies produce optimal staining in paraffin sections from autopsy brain tissue of patients with Huntington's disease, Alzheimer's disease or controls, fixed in room temperature buffered formalin for at least 2 weeks:

Antibody	Type	Antigen	Source	Dilution
SMI-31	mono	phosph. NF	Sternberger-Monocl	1:100,000
10-D5	poly	Aß	Athena Neurosci	1:10,000
MAB1510	mono	ubiquitin	Chemicon	1:30,000
AB748	poly	collagen IV	Chemicon	1:100,000
N1506	poly	GFAP	Dako	1:300

Supplier's recommendations for dilution include: SMI-31 1:1000, AB748 1:320, N1506 1:1. Wet heat pretreatment for Aß substitutes for formic acid pretreatment. These methods promise to allow a wider research use of routinely prepared paraffin-embedded brain tissue.

709.2

APPLICATION OF FLOW CYTOMETRIC TECHNIQUES FOR PURIFICATION OF HUMAN FETAL BRAIN NEURAL POPULATIONS. A. Hassankhani*, R. Rozental, F.-C. Chiu, M. Urban, C. Bassallo and D.C. Spray. Depts. Neurosci. and Neurol., A. Einstein Coll. Med., Bronx, NY 10461, I. Biophysics, Fed. Univ. of Rio de Janeiro, Brazil.

We have established high density primary cultures of neuronal cells from second trimester human fetal brains (*J. Neurosci. Res.*, 1994). Here, we report that these neural cultures can be dissociated to single-cell suspensions, sorted (PS) by size using flow cytometry (low angle forward and side light scattering) and re-seeded to yield cultures selectively enriched for neuronal and glial populations. Neural cell analysis was initially performed on a FACScan and cell sorting was performed using the FACSstar Plus cell sorter. With our methods, two different regions (REG1 and REG2) could be simultaneously defined by electrostatic deflection on the FACStar Plus. Sorted neurons under REG1 were highly homogeneous and viable. As early as 3 hrs-PS, 10% of the cells evaluated proceeded to elaborate processes. Somatal area was ~ 30 μm^2 and lengths of the processes measured were ~ 15 μm. By 1 DIV-PS, numerous long processes were elaborated. By 3 DIV-PS, most of the small cells were process-bearing and all were glutamic acid decarboxylase (GAD)+, neurofilament protein-66 (NF-66)+ and glial fibrillary acid protein (GFAP)-negative. It appeared that these small neurons did not proliferate. By 6 DIV-PS, analyzed somatal area revealed a single component of about 50 μm^2 and lengths of the longest processes were ~ 46 μm. These cells expressed inward currents by 3-6 DIV-PS and spontaneous electrical activity after 10 DIV-PS. In parallel, larger cells with somatal area > 100 μ^2 were recovered under REG 2 (FACS chart). These cells were GFAP+, NF-66 negative and were not excitable. Homogeneous cell populations form human brain are particular interesting for the evaluation of neural dysfunctions that can not be studied in animal models.

709.3

DEVELOPMENT AND CHARACTERIZATION OF TWO HISTOCHEMICAL TECHNIQUES FOR THE RESPECTIVE DETECTION OF NEURONAL AND MYELIN DEGENERATION. L.C. Schmued*, A.C. Scallet, S. Ali, Z. Binienda, J. Bowyer, and W. Slikker Jr. Div. of Neurotoxicology, National Center for Toxicological Research /FDA, Jefferson AR, 72079.

Two techniques have been developed which allow for the rapid and sensitive localization of either neuronal or myelin degeneration following insults by a variety of neurotoxicants. Different classes of neurotoxicants used include excitotoxins (domoic or kainic acid), oxidative free-radical generators (iron or manganese salts), inhibitors of mitochondrial respiration (3-nitropropionic acid), and pyridoxal phosphate/GABA depleting agents (isoniazid). Neuronal degeneration was demonstrated by the use of fluorescent dyes which labeled the cell bodies and proximal dendrites of degenerating neurons. This labeling pattern was generally comparable to that seen using conventional suppressed silver methodologies. To facilitate multiple labeling studies, green (Fluoro-Jade) and red (Fluoro-Ruby II) fluorochromes were developed. These probes can be combined with fluorescent antibodies, apoptosis markers, axonally transported tracers, or counterstains of complimentary emission color to simultaneously reveal the degeneration status along with the anatomy, cytochemical content, chromosome fragmentation, or the connectivity of identified neurons. Degeneration of the myelin sheath was demonstrated by incubating the tissue in a heated, buffered, gold chloride solution. Depending on the nature and duration of toxicant exposure, myelin degeneration could be characterized by a swelling or fragmentation of the myelin sheath, the presence of myelin containing macrophages, or the absence of normal myelin staining. The aforementioned two methods provide a simple, reliable, rapid and sensitive means for detection of neuronal or myelin degeneration following exposure to different classes of neurotoxicants.

709.4

CONVENTIONAL AND FIELD EMISSION HIGH RESOLUTION SCANNING ELECTRON MICROSCOPY OF CEREBELLAR SYNAPTIC JUNCTIONS. O.J.Castejón*. Instituto de Investigaciones Biológicas. Facultad de Medicina. Universidad del Zulia. Maracaibo, Venezuela.

Teleost fishes, primate and human cerebelli were processed for conventional (CSEM) and field emission high resolution scanning electron microscopy (FEHRSEM) to study the outer and inner surfaces of axodendritic, glomerular and axosomatic synapses. Axodendritic junctions of climbing fibers and Golgi axonal ramifications were studied in gold-palladium and chromium coated samples. CSEM of cryofractured glomerular synapses exhibited the outer surface view of "en passant" mossy fiber glomeruli and the inner view of transversally and sagitally fractured glomeruli. The cryofracture method exposed the axosomatic contacts of basket axonal collaterals upon the Purkinje cell and the climbing fiber bulbous endings upon the Golgi cell. FEHRSEM of parallel fiber-Purkinje spines showed the three-dimensional structure of the synaptic membrane complex. The spheroidal synaptic vesicles appeared embedded in a homogeneous axoplasmic substance. Round subunits, 15-20 nm in diameter, were observed at the postsynaptic membrane and the postsynaptic density, presumably related to neurotransmitter receptors.

709.5

MEASUREMENT PROBLEMS ASSOCIATED WITH THE RECONSTRUCTION OF SYNAPTIC STRUCTURES AT THE ELECTRON MICROSCOPIC LEVEL. **R.L. Cooper*, A. Feuerverger[1], M. Menzinger[2], L. Marin and H.L. Atwood. Depts. of Physiology, Statistics[1], and Chemistry[2], Univ. of Toronto, Toronto, Canada**

Quantitative structural information on synaptic components, including synaptic vesicle diameters, synaptic area, and dimensions of the presynaptic dense bodies of 'active zones', is commonly obtained from ultrathin sections visualized with transmission electron microscopy. Consideration of the loss of structural information resulting from projection of a section of a three-dimensional structure onto a two-dimensional photographic plate is necessary. The relation between the true size distribution and the theoretical and actually observed size distributions of projected images is discussed for the case of a population of spherical synaptic vesicles. The observed distribution is demonstrably degraded due to the non-observation of optically thin sections of small radius. Consideration of the degradation of the observed projected images is used to devise a method for interpretating the observed distribution in terms of the true distribution. This approach provides a more rational measure of the population mean and coefficient of variation. For other, non-spherical, synaptic structures (dense bodies and synaptic area), available information on the uncertainties of the observations is used to determine methods for best estimates of the true structural dimensions. (Supported by Medical Research Council, NeuroScience Network, & Natural Sciences and Engineering Council , Canada).

709.6

HOMOLOGUE OF *DROSOPHILA* NEURAL PROTEIN FREQUENIN SELECTIVELY EXPRESSED IN CRUSTACEAN PHASIC MOTOR TERMINALS. **H. L. Atwood[1]*, M Msghina[1], H. Lindemeier[2], and O. Pongs[2]. [1]Department of Physiology, University of Toronto, Toronto, Ontario, CA M5S 1A8, and [2]Zentrum für Molekulare Neurobiologie, Institut für Neurale Signalverarbeitung, D-20246 Hamburg 20, FRG**

Two types of nerve terminal with markedly differing release characteristics, classified as phasic and tonic, have been described in excitatory motor axons of crustaceans. Phasic terminals have high initial quantal output, but show less facilitation and more depression than tonic terminals (Atwood and Wojtowicz, 1986, Int. Rev. Neurobiol. **28**, 275-362). Individual crayfish muscles may be innervated solely by phasic or tonic motor axons, or by both types. Advantage was taken of this to study the expression of the Ca^{2+}-binding protein frequenin (frq; 21 KD), which has been implicated in modulation of synaptic facilitation (Pongs et al., 1993, Neuron **11**, 15-28). Affinity purified monospecific polyclonal anti-frq antibody (1:50), raised against *Drosophila* frequenin, was used in conjunction with FITC conjugated secondary antibody (1:200). Terminals were visualized with a Bio-Rad scanning confocal laser microscope and a Nikon 40x water immersion lens. For comparison, preparations were stained with the vital dye 4-Di-2-Asp, which strongly labels both types of terminal.

In all preparations tested, antibody reaction was strongest in phasic terminals, and absent or much weaker in tonic ones. Thus, there was a staining reaction in terminals innervating the superficial abdominal extensor muscle (purely phasic), weak reaction in the leg opener muscle (purely tonic), and selective staining of phasic terminals in the dually innervated leg extensor muscle. In the superficial abdominal flexor muscle (believed to be purely tonic) there was selective reaction in the terminals of one of the six axons. In all preparations, the main axon and preterminal axon branches showed only faint reaction. Our results indicate that frequenin, or a closely related protein, is enriched in crayfish phasic nerve endings that have high initial quantal output. (Supported by MRC Canada and Faculty of Medicine, Univ. of Toronto)

709.7

COMPARISON OF THE FM1-43 AND SV2 STAINING PATTERNS OF AMPHIBIAN NEUROMUSCULAR JUNCTIONS. A. W. Everett, S. J. Packard and L. Beazley*. Department of Physiology, University of Western Australia, Nedlands 6907, Australia.

The styryl dye FM1-43 labels nerve terminals in an activity dependent manner, purportedly by becoming trapped in recycled synaptic vesicle membranes at transmitter release sites in the terminal. We therefore compared the distribution FM1-43 labelled terminals with those labelled with an antibody to the SV2 antigen of synaptic vesicle membranes. Toads (*Bufo marinus*) were sacrificed by pithing and the iliofibularis was pinned-out *in vitro*. FM1-43 at 2 μM in Ringer containing 60 mM KCl was added to the muscle for 5 minutes. The muscle was then washed briefly and fixed. Other muscles were fixed for staining with the SV2 antibody. Terminals on teased muscle fibres were imaged by fluorescence confocal microscopy. FM1-43 staining appeared as spots or more often as bands along the length of terminal branches at a frequency of not less than 59/100 μm; some larger spots probably consisted of several bands that could not be clearly resolved. SV2 staining was similarly punctate with spots occurring at a frequency of not less than 40/100 μm of terminal branch; individual spots were generally larger than FM1-43 stained spots and less clearly defined. Furthermore, both staining procedures revealed very large differences in the intensity of staining of spots within single branches. The results support the notion that FM1-43 labels synaptic vesicles concentrated at release sites in a nerve terminal and that variation in the uptake of the dye at these sites may reflect differences in the number of vesicles or the composition of their membranes.

709.8

A 3D CONFOCAL STUDY OF TYPE-IDENTIFIED NEUROMUSCULAR JUNCTIONS. Y.S. Prakash*, S.M. Miller and G.C. Sieck. Depts. of Anesthesiology, Physiology and Biophysics. Mayo Foundation, Rochester, MN 55905.

We used a novel three-color fluorescence immunocytochemical technique to simultaneously visualize innervating axons and nerve terminals, motor endplates, and muscle fiber type in the rat diaphragm. Nerve terminals and axons were labelled with an antibody to protein gene product 9.5 (PGP), endplates with a-bungarotoxin, and muscle fibers with an antibody to fast (type II) skeletal myosin. Fluorescein (nerve terminal), tetramethylrhodamine (endplate) and Cy5 (type II muscle fibers) were used as fluorescent dyes to distinguish the three structures. The triple-labelled neuromuscular junctions (NMJs) were optically sectioned using a confocal microscope, and 3D views of the NMJs were created. Both 2D and 3D morphological parameters such as axonal diameter, terminal and endplate planar and surface areas, endplate gutter depth, and extent of overlap of terminal and endplate, were extracted from these images. The results indicate that 1) in general, larger axons innervate larger muscle fibers; 2) when normalized for fiber diameter, planar and surface areas of both terminals and endplates are larger on type I fibers; 3) NMJs on type II fibers generally appear more complex; and 4) the extent of overlap is greater in type I fibers. These morphological differences may underlie fiber type differences in neuromuscular transmission. Supported by NIH grants HL34817 and HL37680.

709.9

IMMUNODETECTION OF BOTULINUM TOXIN A (Bot A) ON ITS TARGET MEMBRANE TO SHOW BINDING SITES AT THE LIGHT MICROSCOPIC LEVEL. G.A. Canziani and R.D. Crosland*. Toxinology Div., U.S. Army Med. Res. Inst. Infec. Diseases, Frederick, MD 21702.

The most potent toxins known are produced by strains of *Clostridium botulinum*. To paralyze the vertebrate neuromuscular junction the toxin must attach to the nerve endings, be translocated into the terminal, and after activation of its enzymatic activity, hydrolyze proteins of the exocytotic apparatus. Our goal was to develop a convenient, reliable technique to detect specific binding of Bot A to its targets. Our technique utilized fluorescently labeled latex spheres, which are capable of detecting a single receptor.

Non-specific binding sites on 7 μm thick sections of unfixed, cryosectioned mouse diaphragm were first blocked with 20% goat serum in PBS (GS/PBS). We incubated the diaphragm for 1 h at RT with various concentrations of Bot A in GS/PBS, followed by incubation with rabbit anti-Bot A antibody, biotin-labeled goat anti-rabbit antibody, and finally avidin-labeled, 0.03 μm diameter, fluorescent latex spheres. As expected, binding was limited to the presynaptic membrane. The technique allowed us to visualize with light microscopy a minimum of 6 Bot A binding sites per μm^2 of nerve ending surface. In addition, we could detect binding on diaphragms that were exposed to as little as 10 pM Bot A, which is in the low range of effective *in vitro* doses that block twitch tension. This a convenient technique for detecting Bot A receptors and may be applicable to other receptors as well.

709.10

ASSESSMENT OF DIABETIC NEUROPATHY BY QUANTITATION OF NERVES IN HUMAN SKIN BIOPSY. W.R. Kennedy,, G. Wendelschafer-Crabb, U of MN, Minneapolis, MN 55455, and C. K. Knox,*, Minnesota Datametrics Corporation, St. Paul, MN 55126

Evaluation of nerves in skin biopsy yields valuable information about peripheral nerve endings. Comparison of nerves in skin from normal vs. diabetic donors reveals striking differences in the pattern of innervation as well as the amount of nerve present in various structures of the skin.

Biopsies obtained from normal and diabetic donors (with informed consent), were fixed, sectioned and processed for double-stain immunohistochemistry using antibodies to PGP 9.5 to localize nerves and type IV collagen to localize basement membrane. Digitized image files were obtained using a laser scanning confocal microscope. Lengthes and volume of nerve as a function of the volume of epidermis or sweat gland were determined by computer analysis and quantification of three dimensional reconstructions.

Nerves in skin can be clearly visualized using antibody to PGP 9.5. The relationship of nerve to other structures can be correlated by double staining with type IV collagen. The amount of nerve was greatly reduced or absent in the biopsies from diabetic donors as compared with controls. A similar decrease in numbers was seen in the sudomotor nerves of sweat glands in biopsies of diabetic subjects. The data clearly indicate that skin biopsy evaluation is useful in the clinical assessment of neuropathy. Staging of neuropathies from skin biopsy data will be useful in assessing the effects of treatments such as drug therapy and pancreas transplantation.

GENE STRUCTURE AND FUNCTION V

710.1

STABILITY OF TAU mRNA IN PC12 CELLS IS MEDIATED BY *cis*-ACTING SIGNALS, LOCATED IN THE 3'UTR OF THE MESSAGE. I. Ginzburg*, E. Sadot, R. Marx-Rattner and J. Barg. Dept. of Neurobiology, Weizmann Institute of Science (WIS), 76100 Rehovot, Israel

Induction of neurite outgrowth in PC12 cells is transcription-dependent and is associated with the accumulation of tau proteins. The increase in tau proteins levels, results from an increase of tau mRNA levels which reflects increased transcription and/or stability of the message.The latter possibility was tested in NGF induced PC12 cells. We found that the half life of tau-mRNA is elevated to 18 hours in induced cells as compared to 7 hours in uninduced cells.To identify tau sequences involved in mRNA stabilization, selected fragments of the 3'UTR were subcloned to the 3' end of the coding region of human c-fos, used as a reporter gene. The transfected cell lines overexpressing c-fos constructs did not show any morphological differences following NGF induction. Stable PC12 line containing construct B (about 1400 nucleotides of the 3'UTR), show a significant increase of 6 folds in the steady state c-fos mRNA levels following NGF induction. The results demonstrated that the half-life of fos-B mRNA increases to 2.1 hrs as compared with 1 hr in cells transfected with c-fos alone. The half-life of fos-B mRNA is even longer when transfected cells are treated with NGF>5.8 hrs. The effect is neuronal specific, as it is not observed in 3T3 cells transfected with the same constructs. By using a uv-cross linking assay a specific binding of proteins prepared from neuronal cells was observed while no binding activity was detected when extracts were prepared from 3T3 cells. Thus we conclude that tau mRNA stabilization is contributed by cis-signals located at the 3'UTR of the message in conjunction with trans-acting neuronal proteins. *[Supported by the Israel Academy of Sciences and Humanities, the GIF Foundation and the Forschheimer Center for Molecular Genetics (WIS)].*

710.2

CHARACTERIZATION OF IMMORTALIZED PINEAL CELL LINES DERIVED FROM TRANSGENIC MICE BY TARGETED TUMORIGENESIS AND ITS USE FOR THE STUDY OF TRYPTOPHAN HYDROXYLASE (TPH) GENE REGULATION. J.H.Chung*, S.O.Huh, Y.I.Chung, D.H.Park, T.H.Joh AND J.H.Son. Cornell University Medical College at The W.M.Burke Medical Research Institute, White Plains NY, 10605.

The lack of immortalized serotonergic cell lines has been a major obstacle to the study of the tissue-specific differential regulation of tryptophan hydroxylase (TPH) gene expression in the pineal gland during circadian rhythm. TPH is the first step enzyme in serotonin and melatonin biosynthesis in neuroendocrine cells of the pineal gland. Previously, we demonstrated that a 6.1 kb 5' upstream region of the mouse TPH gene directs the restricted expression of lacZ reporter gene expression to the pineal gland and the median and dorsal raphe nuclei in transgenic mice. Therefore, to develop TPH-expressing pineal cell lines we first established transgenic mice carrying a construct consisting of 6.1 kb of 5' flanking region fused to the SV40 T-antigen. These animals developed highly invasive pineal tumors and died at 12 to 15 weeks of age. The pineal tumors obtained from the transgenic mice were utilized to establish the immortalized pinealocyte-derived cell lines, PGT-811 and PGT-β, which exhibit characteristic properties of the pinealocyte, such as TPH and N-Acetyltransferase activities. Using PGT-β cells transient transfection analysis revealed cAMP responsive, tissue-specific enhancing and repressing elements in the 5' upstream region of the mouse TPH gene.

710.3

DNA Mismatch Repair and DNA Methylation in the Adult Rat Brain P.J. Brooks*, Cheryl Marietta, and David Goldman, Sect. on Mol Neurobiology, Laboratory of Neurogenetics, NIAAA/NIH

DNA repair is essential to maintaining the integrity of the nucleotide sequence of cellular DNA over time. While much data has accumulated recently on the mechanisms of DNA repair, little is known about the DNA repair capacity of cells in the adult brain. Such knowledge is important in view of the neurological deficits seen in individuals with DNA repair diseases such as Cockayne's Syndrome and Xeroderma Pigmentosum. In the present study, we have investigated the capacity of nuclear extracts from adult rodent brain neurons to carry out DNA mismatch repair. We focused on the repair of G:T and G:U mismatches, which arise from the spontaneous deamination of 5-methyl cytosine (5-MeC) to thymine, or cytosine (C) to uracil, respectively, as these are the only types of DNA mismatches that can arise in nondividing cells. Deamination of both 5-MeC and C occur spontaneously at an estimated rate of 1-10 (5-MeC) or 100-500 (C) times per day per human genome. We found that nuclear extracts from adult brain neurons can correct G:T and G:U mismatches back to GC base pairs. Several other types of DNA mismatches could not be processed. In brain nuclear extracts, the G:U repair system was much more active than the G:T repair system. These data provide the first direct demonstration that neurons in the adult mammalian brain have the capability to carry out specific types of DNA mismatch repair.

We previously reported (Nsci. Abst 20: 100.6) that the adult brain contains high levels of DNA methyltransferase activity. We propose that one function of DNA methyltransferase in the adult brain is to remethylate newly incorporated cytosine residues from G:T mismatch repair following deamination of 5-methyl cytosine, thereby maintaining the original pattern of DNA methylation.

710.4

TRIPLET - REPEAT DNA: UNSTABLE REGULATORY GENES COULD BE EVOLUTIONARY "TUNING-KNOBS". D. G. King*, Department of Anatomy and Department of Zoology, Southern Illinois University at Carbondale, Carbondale, IL 62901.

Excessive expansion of triplet repeat DNA sequences causes several neurological diseases (Martin, *Science* 262:674). But the length of these mutationally unstable sequences also modulates normal gene transcription activity (Gerber et al., *Science* 263:808), as predicted from the basic principle that site-specific mutation can offer significant evolutionary benefits (King, *Science* 263:595). The concept that highly mutable genes can be adaptively advantageous has not yet been widely applied in genetic neurobiology, presumably because it contradicts the common opinion that mutation in any form is almost always deleterious. In computer simulations, however, deterministic selection at a single locus can favor unstable alleles, if the quantitative phenotypic effects of individual mutations are small and if competing Mendelian alleles are not optimally fit, especially if the adaptive peak fluctuates over time. Natural selection might similarly favor mutation-prone genes if the usual consequences of mutation were suitably constrained. Regulatory genes based on unstable triplet-repeat length could thus function as evolutionary "tuning-knobs", efficiently establishing adaptive genotypes in a shifting environment. This idea may offer a useful paradigm for studying patterns of genomic organization underlying innate but evolutionarily flexible patterns of behavior.

710.5

IDENTIFICATION OF A REGION IMPORTANT FOR MAO-B SUBSTRATE AND INHIBITOR SELECTIVITY J. Grimsby*, M. Zentner and J.C. Shih. Dept. of Mol. Pharmacol. and Toxicol. Sch. of Pharmacy, Univ. of So. Calif., 1985 Zonal Ave., Los Angeles, CA 90033.

Monoamine oxidase (MAO) A and B are flavoenzymes that catalyze the oxidative deamination of biogenic and xenobiotic amines. The MAO isoenzymes are defined by their substrate and inhibitor selectivity. MAO-A preferentially oxidizes serotonin (5-HT), whereas MAO-B preferentially oxidizes phenylethylamine (PEA). To search for domains that confer substrate and inhibitor selectivities two chimeric proteins were constructed and expressed in yeast. Replacement of a MAO-A segment (residues 161-375) with the corresponding region of MAO-B, termed $AB_{161-375}A$, converted typical MAO-A catalytic properties to MAO-B like ones. Similar to wild-type MAO-B, $AB_{161-375}A$ oxidizes PEA (V_{max} 32±5 nmol/20 min/mg protein, Km 23±4 μM) but not 5-HT. The IC50 value for deprenyl is 20 fold less than the IC50 for clorgyline. However, the reciprocal chimera in which a MAO-B segment was replaced by the corresponding region of MAO-A, termed $BA_{152-366}B$, lacked catalytic activity. The lack of catalytic activity was not due to aberrant expression but rather an inactive protein as demonstrated by Western blot analysis. These results demonstrate that the MAO-B amino acids 152-366 contains a domain(s) that confers substrate and inhibitor selectivity. (Supported by NIMH grants R37 MH39085 (MERIT Award), K05 MH00796 (Research Scientist Award), R01 MH37020 and Welin Professorship).

710.6

SEQUENCE PREFERENCE OF MOUSE HISTONE H1° PROTEIN EXPRESSED IN E. *COLI* CELLS Duan Su, Philip G Rhodes* and Susan E. Wellman. Dept. Pharmacol.& Toxicol., Univ. MS Med. Ctr., Jackson, MS 39216

Histones are involved in packaging DNA in chromatin and maintaining the supernucleosome structure. When histones bind DNA, they may make DNA less accessible. The histone H1° exists mainly in quiescent or terminally differentiated tissue. To discover if histone has a DNA sequence preference will help to understand its cellular function.

The cloned mouse histone H1° protein expressed in E. *coli* cells has a sequence preference when it binds to a 214mer DNA fragment. This 214mer DNA is a fragment of plasmid vector pBR322. Structurally this 214mer DNA can be divided into two region: a GC-rich sequence and a AT-rich sequence. The results of thermal denaturation experiments show that histone H1° protein bound preferentially to the GC-rich region of 214mer DNA. This experiment indicates that the histone H1° protein is not totally non-specific, but rather binds with some sequence preference to DNA.

710.7

PARTIAL CHARACTERIZATION OF A BRAIN SPECIFIC ssDNA-BINDING PROTEIN. *Raghunathan, A., Poornima, T., Mohan C. Vemuri, School of Life Sciences, University of Hyderabad, Hyderabad 500134, India, Dept. of Biology, Wesleyan University, Middletown, CT 06459, USA.

Tissue- or cell-specific gene expression involves an interplay of DNA-protein interactions and DNA binding proteins play a pivotal role, in terms of regulation. This study involves the purification and characterization of a single strand DNA binding protein which is possibly involved in the developmental regulation of rat brain. The protein was purified using DNA-cellulose chromatography and DNA binding properties were confirmed using filter binding and mobility-shift assays. The protein has a molecular weight of 56 kDa with a pI of 5.2 and is denoted as 56 ssDBP. Intrinsic fluoroscence spectral studies suggest the presence of tryptophan in a buried condition and the amino acid analysis shows an abundance of glycine and serine residues. 56 ssDBP has no significant influence on conformation and melting profiles of calf thymus DNA. It showed no influence on DNA polymerase activity suggesting that the protein may not be involved in DNA replication, repair and/or structural organization, leaving scope to its likely involvement in transcriptional regulation. Immunological studies on Western blots indicated its distribution specific to the brain. (Supported by a SRF grant to AR from ICMR, India, and UGC-Research scientist-'B' grant from UGC, India, to MCV).

710.8

SOMATIC GENE DELIVERY TO GENERATE GAIN OF FUNCTION NGF MOSAICISM. B. Mukherjee, A. Brooks, R. Starr, and H.J. Federoff*, Departments of Microbiology, Immunology and Neurology, University of Rochester School of Medicine, Rochester, NY 14642.

To investigate NGF action in specific regions of the adult mammalian nervous system we are developing a somatic gene transfer approach to create genetic mosaics in mice. In this approach we use a binary system that involves an inactive germline-transmitted transgene that is activated by the somatic delivery and expression of cre recombinase. We have constructed an excision activated transgene (XAT) that is composed of the rat NSE promoter, an inactivating cassette, a bicistronic transcription unit consisting of a NGF minigene and IRES initiated reporter gene (NSEprNGFXAT). Introduction of the NSEprNGFXAT into *E. coli* that constitutively express cre recombinase resulted in removal of the inactivating cassette in 100% of clones. By constrast, no excisional process occurred in bacteria that did not express the recombinase gene. The NSEprNGFXAT has also been introduced into cell lines and transgenic mice. Several cell lines and transgenic lines of mice express the inactive NGF transcript. A defective herpes amplicon vector transducing the cre recombinase gene (HSVcre) has been constructed as the somatic gene delivery vehicle. Infection of cell lines, primary neurons isolated from transgenic animals, and transgenic mice is being used to produce activation of the NSEprNGFXAT. Studies are examining the efficiency of gene activation, levels of NGF and reporter gene produced, and the biological effects produced by patches of mosaic tissue.

710.9

AAV-VECTOR MEDIATED HUMAN HSP72 GENE TRANSFER INTO CULTURED CELLS. C. Lippman*, F. Welsh, M. Kaplitt, W. O'Connor, M. During, C. Mobbs, M. O'Connor, A. Freese. Div. Neurosurg. Univ. PA., Phila., PA 19104; Graduate Hosp., Phila., PA 19146; Lab. Neurosci. Rockefeller Univ., New York, NY 10021 .

Heat shock protein 72 (hsp72) expression is associated with increased neuronal survival after a variety of stresses. A causal relationship between this expression and neuronal protection has not been demonstrated. Introduction into the CNS of the gene encoding hsp72 resulting in expression may permit the assessment of such a relationship.

We have developed an adeno-associated viral (AAV) vector with the human hsp72 gene under control of a cytomegalovirus promoter (AAV-hsp72). When introduced into rat cortical or striatal mixed neuronal-glial cultures, the vector directed both short- and long-term hsp72 expression.

At three days, cultures were exposed to AAV-hsp72, AAV-LacZ (a control vector containing the LacZ marker gene), or PBS. Hsp72 expression was qualitatively measured after two or seven days of viral vector incubation. Using a monoclonal antibody against human hsp72, immunocytochemistry was performed. Only those cultures transfected with AAV-hsp72 demonstrated staining for hsp72, while staining for ß-galactosidase was observed in cultures transfected with AAV-LacZ, but not in cultures transfected with AAV-hsp72 or PBS. Western blot analysis showed similar results, with inducible hsp72 protein only present in those cultures transfected with AAV-hsp72. Absence of cytotoxicity was confirmed by a number of techniques.

These results demonstrate that this vector may be used to introduce the human hsp72 gene into CNS neurons with subsequent transgene expression. We are evaluating the neuroprotective effect of this vector and implications for therapy in stroke and trauma.

710.10

DEVELOPMENT OF VIRUS VECTORS FOR GENE TRANSFER INTO NEURONS: ANALYSES OF PROMOTER FUNCTION. S. J. Davis, J. Schaack, R. L. Smith and C. L. Wilcox* Depts. of Anatomy and Microbiology, Colo. State Univ., Ft. Collins, CO 80523, and Depts. of Microbiology and Neurology, Univ. of Colo. Health Sci. Ctr., Denver, CO 80262.

The ability to transfer genes into neurons has important utility in the study of neuronal physiology and gene regulation, and for gene therapy in the treatment of diseases of the nervous system. Replication-defective, recombinant adenovirus vectors have been used to infect neurons without evidence of pathogenicity. Development of virus vectors to provide cell-type-specific promoter regulation is important for many applications. We have constructed replication-defective recombinant adenovirus vectors to investigate the regulation of several promoters using the reporter *lacZ* gene to express ß-galactosidase. Initial studies have utilized rat dorsal root ganglia neurons in tissue culture to examine expression from several promoters. An adenovirus vector containing the cytomeglovirus immediate early gene promoter produced expression of ß-galactosidase in greater than 90% of the neurons 24 hr postinfection, with continued ß-galactosidase expression for 30 days without evidence of cytotoxicity. Infection with an adenovirus vector containing the adenovirus early E1A promoter driving *lacZ* resulted in ß-galactosidase expression in neurons. Use of the E1A promoter provides an expression system that is not active in the cell line required to package the adenovirus vector, thus allowing construction of vectors containing toxic genes.

710.11

EXPRESSION OF LACZ GENE USING A HERPES SIMPLEX VIRAL VECTOR RETROGRADELY TRANSPORTED INTO HIPPOCAMPAL GRANULE CELLS IN VIVO. P.A. Serrano*, R.L. Neve[1] & A. Routtenberg. Cresap Neuroscience Laboratory, Northwestern Univ., Evanston, IL 60208; [1]Harvard Medical School and Mclean Hospital, Belmont, MA 02178.

The herpes simplex virus (HSV-1) is used here to introduce foreign genes into postmitotic neurons. The HSV-1 mutant is replication incompetent not entering the lytic cycle (TINS, 1991, 14:428), spreads minimally through brain parenchyma, and is taken up by terminals in the region of the injection site. We determined the extent to which the IE 4/5 promoter of HSV-1 would activate the lacZ gene when introduced into rat hippocampal granule cells.

Injection targeted for mossy fiber terminals in the dentate hilus were guided by a laminar profile of the dentate gyrus constructed from single pulses to perforant path. The population spike and EPSP evoked by stimulation of the perforant path were recorded by an HSV-1-filled micropipette electrophysiologically guided to the dentate hilus. Ejection volumes of 0.1-1.0µl were delivered over a 45-60 min period by directly monitoring the movement of the solution meniscus in the micropipette. HSVlacZ injected into the hilus was retrogradely transported to granule cell bodies. We observed robust expression of ß-galactosidase in granule cell bodies on both the dorsal and ventral leaf 24-72h after a single injection. Dendritic trees of granule cells were filled with ß-galactosidase and mossy fiber axons could be traced to their terminal zone in stratum lucidum. Axons of mossy fibers showed the characteristic rosettes of mossy fiber terminals. CA3c pyramidal cells in the hilus did not translate lacZ. HSV-1 is a highly effective vector for introducing foreign genes into hippocampal granule cells in vivo and in combination with electrophysiological guidance can restrict expression to a particular cell type. Supported by NIMH grant MH25281-2 to A.R., NSF Post-doctoral grant to P.A.S., and HD24236 grant to R.L.N.

710.12

RECOMBINANT VACCINIA VIRUS AS A TOOL TO OVER-EXPRESS PROTEINS IN CULTURED HIPPOCAMPAL NEURONS. T. Koothan*, M. Maletic-Savatic and R. Malinow. Jones Bldg, Cold Spring Harbor Lab., Cold Spring Harbor, NY.

Recombinant vaccinia viruses have been successfully used to over-express genes of interest in several mammalian cell lines and in rat hippocampal slices. Here we describe a method to over-express proteins of interest in cultured rat hippocampal neurons using the vaccinia virus system. Neurons cultured separately, as described by Banker, did not express the recombinant protein of interest following vaccinia virus infection. In contrast, co-cultures of hippocampal neurons and astrocytes survive and express beta-galactosidase protein following infection with recombinant vaccinia virus. The percentage of infected neurons depends upon the density of the astrocytes co-cultured along with neurons. Recombinant vaccinia virus carrying rat alpha-CamK II have been used to infect cultured hippocampal neurons. We discuss the localization of CamK II in the neurons following infection.

710.13

EXPRESSION OF HEAT SHOCK PROTEINS (HSP70 AND HSC70) AND NUCLEAR TRANSLOCATION IN THE RABBIT BRAIN FOLLOWING HYPERTHERMIA. P. Manzerra* and I. R. Brown. Dept. of Zoology, Univ. of Toronto, Scarborough Campus, West Hill, Ont., Canada M1C 1A4.

The heat shock response is characterized by i) an overall decrease in ongoing mRNA and protein synthesis, ii) an induction of heat shock mRNAs and proteins (hsps), iii) nuclear translocation of hsps and iv) collapse of elements of the cytoskeleton in some cell types. We have carried out Western blot and immunocytochemical studies using antibodies which are specific in recognizing constitutive hsc70 protein or stress-inducible hsp70 protein. Large neurons, such as Purkinje neurons and motor neurons of the spinal cord, express high levels of constitutive hsc70 protein, however their induction of hsp70 protein in response to hyperthermia is attenuated compared to the rapid and robust induction which is seen in adjacent glial cells. In the present study we have investigated whether neuronal cell populations exhibit other features of the heat shock response. Following hyperthermia a transient induction of hsp70 protein was observed primarily in non-neuronal cell populations in the rabbit brain (ependymal cells, oligodendrocytes and astrocytes). At 1 hr post-hyperthermia, a redistribution of both hsp70 and hsc70 protein from the cytoplasm into the nucleus occurs in ependymal cells of the choroid plexus with a return to the cytoplasm by 5 hr. Glial cells exhibit nuclear localization of stress-inducible hsp70 followed by a later appearance of the protein in cellular processes. Nuclear translocation of hsc70 protein was not observed in several neuronal cell populations in response to hyperthermia (Purkinje, hippocampal and thalamic neurons), however it was observed in motor neurons of the spinal cord. These neurons showed a translocation of hsc70 protein into the nucleus, followed by exit to the cytoplasm, with a temporal profile similar to that observed for ependymal cells.

710.14

CELLULAR LOCALIZATION OF HEAT SHOCK mRNAS (HSC70 AND HSP70) IN THE RABBIT BRAIN. J.A. Foster* and I.R. Brown. Dept. of Zoology, Univ. of Toronto, Scarborough Campus, West Hill, Ont. Canada, M1C 1A4.

The hsp70 multigene family is comprised of constitutive and hyperthermia-inducible members. Using radioactive *in situ* hybridization we have shown high constitutive expression of hsc70 mRNA in certain neurons. Here we employ non-radioactive *in situ* hybridization to examine cellular distribution of heat shock mRNAs. At high resolution, we observed hsc70 mRNA in the cell bodies of neurons. In cortical neurons (layer 2 and 5), deep cerebellar neurons (DCN), and brain stem neurons (BSN), we also detected hsc70 mRNA in apical dendritic processes. This dendritic distribution of hsc70 mRNA was not detected in hippocampal, some cortical neurons, and most cerebellar neurons. Following heat shock, neurons with the highest levels of constitutive hsc70 mRNA, i.e. BSN and DCN, showed more distal transport of hsc70 mRNA in dendritic processes. By radioactive and non-radioactive methods we do not detect hsc70 mRNA in glial cells. We have shown a strong glial induction of hsp70 mRNA after hyperthermia. For example, oligodendrocytes showed intense signal in cytoplasmic cap regions following 1 hour of hyperthermia. Later time points following heat shock showed this distribution to be more spindle-like but the extent of mRNA in oligodendrocyte processes was not comparable to that observed for hsc70 mRNA in dendritic processes of BSN and DCN. We detected basal levels of hsp70 mRNA in control neurons, particularly when tissue was fixed after freezing; this signal was localized to cell bodies. Following heat shock, an immediate glial induction and a delayed accumulation of hsp70 mRNA in cell bodies of neurons was observed. These data demonstrate that neurons with high constitutive levels of hsc70 mRNA increased dendritic transport of this message following hyperthermia, while the distribution of hsp70 mRNA was localized to cell bodies.

710.15

HETEROGENEITY OF TRANSCRIPTS ENCODING ISCHEMIA-INDUCED HSP70 IN BRAINS OF INDIVIDUAL WISTAR RATS.
Y. Yaida, W. Valentine, K.-S. Kim*, W. A. Pulsinelli and T. S. Nowak, Jr. Dept. of Neurology, University of Tennessee, Memphis, TN 38163.

Phosphorothioate oligonucleotides are more stable than normal phosphodiester oligos in brain, and should therefore be better antisense reagents to modify the postischemic stress response in brain. However, we have had limited success in reducing expression of the inducible 70 kDa stress protein, hsp72, perhaps in part due to heterogeneity of the transcripts encoding this subset of the hsp70 protein family. We have therefore employed a 5' amplification (RACE) method to sequence potential antisense targets in ischemia-induced hsp70 mRNAs. Male Wistar rats were subjected to 10 min 4-vessel occlusion ischemia. After 3 h brains were removed for mRNA preparation or in situ hybridization. The primer for cDNA synthesis consisted of a 30mer previously shown to detect inducible hsp70 mRNAs, which was then ligated to a 23mer from the rat somatostatin promoter, amplified, cloned and sequenced. We obtained the same sequence published for a 2.5 kb transcript from Wistar rats (1), that differs from Sprague-Dawley (SD) sequences (2,3) at positions 6, 9 and 10 bases 5' to the translation start site. However, a 21mer spanning this region of the SD sequence detected an induced mRNA in 30% of Wistar rats, suggesting that the sequences, rather than reflecting strain variation, may be differentially expressed in individual animals. Ongoing studies continue to characterize rat hsp70 mRNAs with the aim of developing suitable antisense reagents.

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710.16

CONCOMITANT EXPRESSION OF INDUCIBLE HSP-70 WITH PROTEIN SYNTHESIS INHIBITION FOLLOWING ISCHEMIA, KAINIC ACID AND HYPERTHERMIA.
A.M. Planas [1*], M.A. Soriano [1,2], E. Rodríguez-Farré[1] and I. Ferrer[2]. [1]Dep. Pharmacol. & Toxicol., CID, CSIC, 08034 Barcelona, and [2]Unitat de Neuropatologia, Hospital Princeps d'Espanya, Universitat de Barcelona, Spain.

Inducible heat shock protein-70 (HSP-70) is expressed in certain regions of the rat brain following hyperthermia, ischemia and kainic acid-induced seizures. These conditions are also known to cause inhibition of protein synthesis. Here we examined induction of hsp-70 mRNA with *in situ* hybridization using a ^{32}P-labelled oligonucleotide probe, and cerebral protein synthesis following *in vivo* i.v. administration of a ^{14}C-labelled neutral amino acid. For each rat, hsp-70 mRNA induction and incorporation of labelled amino acid into proteins were studied in consecutive brain sections. Focal ischemia, hyperthermia and kainic acid caused selective hsp-70 mRNA expression in those brain regions showing decreases in labelled amino acid incorporation into proteins. The level of hsp-70 mRNA expression correlated with the percentage of decrease in labelled amino acid incorporation into proteins in relation to controls. Neither cycloheximide nor anisomycin induced any detectable hsp-70 mRNA expression, despite producing marked protein synthesis inhibition. These observations suggest that hsp-70 mRNA induction and protein synthesis inhibition are two simultaneous events of the cerebral stress response following a variety of insults.

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710.17

MOLECULAR INTERACTION OF NMDA AND DOPAMINE D2L RECEPTORS IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS.
V. D. Nair, H. B. Niznik[1] and R. K. Mishra*. Depts of Psychiatry and Biomedical Sciences, McMaster University, Hamilton, Ontario L8N 3Z5; and Laboratory of Molecular Neurobiology[1] ,The Clark Institute of Psychiatry, Toronto, Ontario, Canada.

The N-methyl D-aspartate (NMDA) receptor, a subtype of glutamate receptor, plays a key role in synaptic plasticity in the central nervous system and dopamine receptors are involved in regulating motor and cognitive behaviours. In the medium-sized spiny neurons of the neostriatum, dopamine modulates neuronal responses mediated by activation of excitatory amino acid receptors. A deficiency in glutamatergic neurotransmission resulting in a disturbed balance between glutamatergic and dopaminergic systems within the neostriatum may play a key role in the pathophysiology of schizophrenia. However the molecular mechanisms underlying this interaction are poorly understood. Using a human neuroblastoma cell line, SH-SY5Y cells, expressing clonal specific human dopamine $D2L_{Long}$ (D2L) receptor, we now demonstrate that transient expression of D2L receptors can be regulated by the inactivation of NMDA receptors. Protein tyrosine kinase inhibitor enhanced the D2L receptor gene activation, whereas inhibition of protein phosphatase 1 or 2A decreased receptor activation induced by NMDA blockade. The study support the possible interaction between NMDA and dopamine D2L receptor. Improved therapy may be obtained by developing drugs that target the molecules which regulate both NMDA and/or dopamine receptors.

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710.18

CELLULAR EXPRESSION OF PIGMENT-DISPERSING HORMONES IN THE BLUE CRAB *CALLINECTES SAPIDUS*.
N. Jaenecke, J. M. Klein, J. Phillips, J. P. Riehm and K. R. Rao*. Dept. of Cellular and Molecular Biology, Univ. of West Florida, Pensacola, FL 32514.

Klein et al. (Biochem. Biophys. Res. Commun. 205, 410-416 (1994)) reported finding two different cDNAs which encoded the precursors of the pigment-dispersing hormones, PDH I and PDH II, in the blue crab *Callinectes sapidus*. In the present studies, these cDNAs have been used to design specific cRNA probes which hybridized selectively with the PDH I- and II-precursor encoding mRNAs.

Northern blot experiments showed that PDH I and II were expressed in eyestalk ganglia; no hybridization products were observed using heart, muscle or hepatopancreas preparations. In situ hybridization experiments showed expression patterns for PDH I and II in the eyestalk of a single animal. Staining of adjacent sections revealed that both PDH isoforms are probably expressed in different cells in the eyestalk. These results suggest different physiological functions of both peptides, as also indicated by the results of assays for melanophorotropic activity in fiddler crabs in which PDH II displayed a 400-fold less potency relative to PDH I.

710.19

EXPRESSION OF ZIF268 IN THE TEMPORAL CORTEX OF MONKEYS RELATED TO LEARNING OF VISUAL STIMULUS-STIMULUS ASSOCIATION.
H. OKUNO*, S. KANOU, W. TOKUYAMA and Y. MIYASHITA. Dept. Physiology, Sch. Medicine, Univ. Tokyo, Hongo, Tokyo 113, Japan.

To investigate the molecular basis of cognitive memory in the primate, we examined expression of immediate early genes in the temporal cortex of monkeys during visual learning. Three Macaque monkeys were trained to perform a pair-association task (PA) that required them to memorize the visual stimulus-stimulus association between computer-generated pictures. Another three monkeys were trained to perform a visual discrimination task (VD) which required them to memorize the stimulus-reward association by using the same set of stimulus pictures as that for the PA task. After training with 12 pairs of stimulus pictures, both PA and VD monkeys were trained using a new set of pictures. Before the monkeys' performance reached a plateau phase (PA, 8-10th session with the new set; VD, 5-6th session), they were perfused immediately after the completion of that day's training session. Serial coronal sections (0.5 mm intervals) of the temporal cortex were stained immunohistochemically. The specificity of the immunoreactivity of each antibody was confirmed by western blotting and by antigen-absorption experiments. We found that Zif268, a transcription factor encoded by an immediate early gene, was expressed in a different manner during PA and VD learning. *Zif268-immunopositive neurons were found to be distributed as patches in the inferior* temporal gyrus in PA monkeys, but not in VD monkeys. The patches included Zif268-immunopositive neurons in the superficial and deep layers as well as in layer IV. Quantification of the immunoreactivity revealed that the Zif268 expression levels in PA monkeys were higher than those in VD monkeys in area 36 ($P = 0.01$, t-test), but not in area 35 and TE. These results suggest that the PA learning activates different gene cascades in the inferior temporal gyrus, particularly in area 36, compared with VD learning, and that Zif268 may play a role in the formation of the visual associative long-term memory in the primate.

710.20

EXPRESSION OF CYTOSKELETAL mRNAs IS ALTERED AFTER AXOTOMY IN MATURE AND AGED F334 RATS.
J.M. Jacob*, B. Srinivasan and A.R. Whitetree. Dept. of Anatomical Sciences, OUHSC, OKC, OK 73190.

Axotomy results in a series of morphologic, biochemical and physiological changes in neuronal cell body. Axotomy also alters structural protein mRNA expression. In this study, the expression of several isoforms of β-tubulin (classes I, II, and IV) as well as the neurofilament triplet protein (NF-L, NF-M, NF-H) was examined in mature (6 mo) and aged (24 mo) F334 rats after axotomy. Fluorogold, a retrograde tracer, was used to locate the motor columns supplying the right sciatic nerve. Rats were killed by decapitation at 12hr, 1d, 3d, 7d or 14d after sciatic nerve transection; spinal cords were removed rapidly and frozen on dry ice. Longitudinal 20 µm sections were made through the lumbar spinal cord region containing the axotomized motor neurons. Using digoxigenin-labeled oligonucleotide or cDNA probes specific for each cytoskeletal protein, levels of mRNA expression were determined. These data suggest that message expression is down-regulated with advancing age, but that expression of cytoskeletal proteins after axotomy in the aging rat follows patterns similar to those seen in the young rat.

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710.21

EXPRESSION OF THE PRION PROTEIN (PrP) GENE IN CULTURED NORMAL HUMAN MUSCLE. E. Sarkozi, V. Askanas, J. McFerrin, and W.K. Engel*. USC Neuromuscular Center, Los Angeles, CA 90017-1969.

Even though abnormal accumulation of PrP has been considered unique to the brain, we have recently demonstrated abnormal accumulation of PrP and increased PrP-mRNA in vacuolated muscle fibers in inclusion-body myositis (reviewed by Askanas et al, 1994). PrP and its mRNA are also increased in human muscle fibers regenerating in vivo, but PrP-mRNA is virtually undetectable in the mature muscle fibers (except focally at the neuromuscular junctions). We have now studied the expression of PrP gene in cultured normal human muscle.

Adult human muscle was cultured aneurally in monolayer from the satellite cells of portions of 5 normal muscle biopsies. PrP-mRNA abundance, relative to 28S ribosomal RNA, was measured at 10, 20 and 40 days of growth by Northern blots, using ^{32}P-labelled cRNA transcribed from a human PrP-cDNA (gift from S.B. Prusiner). To monitor muscle development, creatine kinase (CK) activity was measured in sister cultures. At each stage of growth, cultured human muscle expressed strong PrP-mRNA. Its expression was lowest in 10-day-old cultures, and increased 144% ($p < 0.02$) in 20-day-old cultures. Between days 20 and 40, PrP-mRNA increased only 37% ($p < 0.03$). Between days 10 and 20, increase of CK activity was 166% ($p < 0.04$), similar to the increase of PrP-mRNA; but in contrast to PrP-mRNA, between days 20 and 40 CK activity continued to increase significantly 138% ($p < 0.03$) and linearly. Our study demonstrates for the first time that: 1) the PrP gene is expressed in cultured normal human muscle; and 2) the highest level of its expression occurs during early muscle development. Thus, cultured normal and diseased human muscle should provide an excellent model to study factors influencing synthesis of PrP and its role therein.

LONG-TERM POTENTIATION: PHYSIOLOGY V

711.1

INHIBITION OF LONG-TERM POTENTIATION, BUT NOT LONG-TERM DEPRESSION, BY RYANODINE IN THE RAT DENTATE GYRUS Y Wang, MJ Rowan and R Anwyl* Department of Physiology, Trinity College, Dublin, Ireland

Regulation of Ca release from the intracellular Ca stores of the endoplasmic reticulum (ER) is known to occur in part via the ryanodine receptor, a Ca-activated Ca channel located on the ER. The role of intracellular Ca stores in synaptic plasticity has been studied by investigating the effects of ryanodine on the induction of long-term potentiation (LTP) and long-term depression (LTD). Intracellular patch clamp and field recordings of excitatory postsynaptic potentials and currents (epsps/epscs) were made from the dentate gyrus of hippocamapal slices in response to stimulation of the associational/commissural pathway of rats (~100g). In control recordings, LTP of epscs measuring 180±6%, n=9,(20 min post-stimulation) was induced in response to high frequency stimulation (8 trains each of 8 pulses at 200 Hz, intertrain interval 2 sec). LTD of field epsps and epscs measuring 40±8%, n=5, and 32±5%, n=9 respectively was induced by low frequency stimulation (900 pulses at 1Hz or 2 Hz). In experimental recordings, ryanodine (20 uM) was either perfused extracellularly in experiments recording field epsps, or put in the patch clamp electrode for recording epscs. Ryanodine was found to block LTP, but not LTD. Thus the amplitude of epscs at 20 min post-high frequency stimulation in the presence of ryanodine measured 99±5%,a significant inhibition of LTP. Low frequency stimulation induced LTD of field epsps and epscs measuring 34±6% and 40±8% respectively, values not significantly different from controls.

711.2

CORRELATING DENDRITIC SPINE STRUCTURE WITH FUNCTION BY COMBINED ELECTRON MICROSCOPY AND Ca^{2+} IMAGING. T.H. Murphy*, P.J. Mackenzie, M. Umemiya, and G.S. Kenner, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1Z3

By imaging Ca^{2+} entry associated with miniature excitatory synaptic currents (MESCs) under conditions that favor NMDA receptors (0 Mg^{++}, 5 mM $CaCl_2$, and 1 µM TTX), we have been able to monitor the activation of a single synaptic site. Using this approach we concluded that the amplitude of repeated MESCs at a single synapse (attributed to NMDA receptors) are variable, furthermore some synaptic sites show a higher frequency of MESCs than others. Using cultured cortical neurons that were injected with fura-2 and biocytin, we evaluated the ultrastructural characteristics of dendritic regions that show these miniature synaptic Ca^{2+} transients (MSCTs) by peroxidase staining and serial sectioning. In evaluating the $[Ca^{2+}]_i$ images we identifed a presumed high probability region that exhibited 12 MSCTs from a single ~1 µm^2 dendritic site. Neighboring sites were either inactive or showed only 1 MSCT. Transmission EM revealed that a single large spine synapse (~ 1 µm^2) was centered at the site of MSCT initiation at the presumed high probability synapse. Other synapses were several µm away, making it unlikely that they contributed to the MSCTs. Our results suggest that a high frequency of synaptic responses of varying amplitude can be attributed to a single large synapse.

711.3

Ca^{2+} Signals Associated With The Induction Of Long-Term Potentiation And Long-Term Depression In Pyramidal Cells Of The Rat Visual Cortex. C.Hansel, A.Artola[1] and W.Singer. (SPON: European Neuroscience Association) . Max-Planck-Inst. for Brain Research, Deutschordenstr.46, 60496 Frankfurt, FRG. [1]Present address: Dept. of Neurobiology, ETH-Hoenggerberg, CH-8093 Zürich.

We characterized Ca^{2+} signals evoked by tetanization patterns suitable for the induction of LTP and LTD in layer II/III pyramidal cells of the rat visual cortex using the fluorescent Ca^{2+} indicator fura-2. Neocortical slices (200-250 µm thick) were obtained from rats aged 5-7 weeks. Sharp microelectrode recordings were used to measure responses to stimulation in either layer IV (conditioning pathway) or lateral layer II (control pathway). Three different plasticity patterns were established in cells not filled with the indicator dye. LTD was reliably induced by 5x repeated 50Hz stimulation in layer IV (8 / 9 cells). Application of only one 50Hz burst reduced the success rate in LTD induction to 50% (3 / 6 cells). Pairing of the repetitive 50Hz stimulation with a postsynaptic 20mV-depolarization and lowering of $[Mg^{2+}]_o$ led to reliable induction of LTP (6 / 7 cells). For characterization of evoked Ca^{2+} dynamics, these tetanization patterns were applied to cells filled with fura-2 and changes of dendritic $[Ca^{2+}]_i$ were assessed with single-wavelength excitation at 380nm using an intensified CCD camera. Repetition of 50Hz bursts (LTD pattern) increased burst-induced Ca^{2+} elevations, although Ca^{2+} levels nearly completely recovered to baseline during the 10sec long interburst-intervals (1x50Hz burst: n=12; 5x: n=7). In contrast,Ca^{2+} levels accumulated during application of the LTP inducing tetanization pattern (n=5) to levels higher than those measured during LTD induction. These observations indicate that the induction of LTD and LTP is associated with differences in evoked Ca^{2+} dynamics that are reflected by differences in both amplitude and duration of Ca^{2+} elevations. These results support the assumption that the different thresholds for the induction of LTD and LTP are related to the amplitude and the kinetics of the postsynaptic increase of $[Ca^{2+}]_i$.

711.4

THE INTERPLAY OF DENDRITIC SPINE MORPHOLOGY WITH DEVELOPMENT OF GLUTAMATE RECEPTORS AND VOLTAGE GATED CALCIUM CHANNELS AND ITS IMPLICATION FOR CALCIUM DYNAMICS. S.S. Dalal* and C.E. Niesen. Dept. of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089-1451; and Div. of Neurology, Childrens Hospital of Los Angeles and USC School of Medicine, Los Angeles, CA 90027.

A detailed biophysical computer model that simulates calcium dynamics in dendritic spines in rat hippocampal pyramidal neurons was created. Two features distinguish this model. Whereas previous models have only taken into account calcium efflux from the AMPA and NMDA glutamate receptors (GluRs), the present model accounts for calcium influx into dendritic spines from both GluRs and voltage-gated calcium channels (VGCCs). The existence of N-type VGCCs, within a subset of hippocampal spines, has been confirmed by confocal microscopy (Mills, et al., 1994). The distribution of VGCCs and GluRs on dendritic spines was varied with age from the first postnatal day (PN1) to adulthood as was dendritic spine morphology. Mechanisms that simulate diffusion, pumping, and buffering of calcium were taken from the methods described by Holmes and Levy (Holmes and Levy, 1990). Results from the simulations are in very good agreement with calcium imaging studies in the hippocampal slice (Guthrie, et al., 1991; Jaffe, et al., and Conner, et al., 1994) and other modeling studies (Gold and Bear, 1994; Koch and Zador, 1993), i.e. presynaptic stimulation elevates calcium levels in the spine head to tens of micromoles while, in the dendritic shaft, calcium concentration increases to less than 1 µM. In the adult, both the N-type and the T-type VGCC as well as the NMDA GluR are major contributors to this calcium boost which resulted in LTP. Neither the L-type VGCC nor the AMPA GluR alone could induce LTP. In modeling dendrites less than PN7, the lack of N-type VGCCs and the predominance of headless spines resulted in an absence of LTP. Both NMDA-dependent and NMDA-independent LTP can be induced in the model.

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711.5

INDUCTION OF LTP AND LTD OBSERVED SIMULTANEOUSLY WITH A RISE OF POSTSYNAPTIC CALCIUM IN RAT VISUAL CORTEX.

H. Yasuda and T. Tsumoto* Department of Neurophysiology, Biomedical Research Center, Osaka University Medical School, Suita 565, Japan

An input-associated increase in calcium at postsynaptic sites is suggested to trigger processes for the induction of long-term changes in synaptic efficacy. Furthermore, the extent of this increase is hypothesized to determine the direction of the changes, i.e., a large increase beyond a certain threshold leads to long-term potentiation (LTP) while a small increase below the threshold to long-term depression (LTD). This hypothesis seems testable with microfluorometry using calcium-indicators, such as fura-2 or fluo-3, but so far was not successfully proofed at least in neocortex, probably because these dyes have a strong calcium-chelating action. So, we used another indicator, rhod-2, which has a much weaker chelating action. With this dye, we measured changes in calcium concentration in visual cortical neurons during tetanic synaptic inputs simultaneously with whole-cell current clamp recordings of synaptic activity.

In visual cortical slices of young rats at 9-18 postnatal days, rhod-2 was injected directly into pyramidal cell-like neurons of layer II/III through a patch pipette from which we simultaneously recorded excitatory postsynaptic potentials (EPSPs) of the neuron under observation. Tetanic stimulation of θ-burst type given to layer IV induced a clear increase in fluorescent signal. Usually, this increase was stronger in dendrites than in soma. Also, the decay time of the increased signal was much faster in dendrites than in soma. There was a tendency that tetanic stimulation which led to the induction of LTP of EPSPs induced a higher rise of calcium signal in dendrites than that which led to the induction of LTD.

711.6

DISRUPTION OF A CYCLIC-ADP-RIBOSE SIGNALING PATHWAY INTERFERES WITH LONG TERM POTENTIATION IN AREA CA1 OF THE RAT HIPPOCAMPUS. N.K. Mahanty*, M.E. Gurnack+, T. Walseth+, E.M. Schuman. Division of Biology 216-76, California Institute of Technology, Pasadena CA 91125, +Department of Pharmacology, University of Minnesota, Minneapolis MN 55455.

A recently identified endogenous ligand for the ryanodine-sensitive Ca^{2+} store is cyclic ADP-ribose (cADPr). The enzyme responsible for cADPr synthesis, an ADP ribosyl-cyclase, is stimulated by cGMP. Biochemical assays indicate that the hippocampus has ADP-ribosyl cyclase activity localized in the particulate fraction. The diffusible signal nitric oxide, NO, can stimulate cGMP production by activating a soluble guanlyl cyclase, raising the possibility that the NO generated during the onset of LTP induction may initiate a signal transduction cascade that eventually includes cADPr activation of ryanodine-sensitive Ca^{2+} stores. We have tested this idea by utilizing a competitive antagonist, 8-NH_2-cADPr, of the cADPr binding site on the ryanodine sensitive store. The inhibitor was introduced postsynaptically in a patch electrode into an individual CA1 cell in the acute hippocampal slice. Two independent Schaffer Collateral afferent pathways were alternately stimulated (0.03 Hz) and the evoked excitatory postsynaptic currents, (EPSCs) were recorded. After a 20 minute period of baseline stimulation, during which time the inhibitor was allowed to diffuse into the cell, LTP was induced by pairing depolarization with low frequency (1 Hz) stimulation of one pathway. Following the pairing of depolarization and 1 Hz stimulation a slowly decaying potentiation was observed which typically returned to baseline values within 20 min [mean % of baseline = 99.1 +/- 1.3]. This short-term potentiation was followed by a slow onset depression of approximately 50% which reached asymptote 40 minutes after LTP induction [mean % of baseline = 45.5 +/- 0.5]. Control slices or slices injected with boiled 8NH_2-cADPr exhibited normal LTP [mean % of baseline = 169.4 +/-2.1 and 157.3 +/-1.4]. These data suggest that a cADPr signaling pathway maybe involved hippocampal synaptic plasticity and may participate in determining whether synapses become potentiated or depressed during induction. This work supported by USPHS T 32 DA07097 (M.E.G.), DAO5695 (T.W.) and NS32792 (E.M.S.)

711.7

MODELS OF CALCIUM BINDING TO KINETICALLY DISTINCT SITES ON CALMODULIN: IMPLICATIONS FOR LTP. O. V. Yousukhno*[1], W. R. Holmes[2] & W. B Levy[1]. [1]Univ. of Virginia Health Sciences Ctr., Dept. of Neurosurgery, Charlottesville, VA 22908 and [2]Dept. of Biological Sciences, Ohio University, Athens, OH 45701

We have revisited the Holmes & Levy (1990) biophysical model of associative LTP. Again we consider a morphologically correct granule cell of the dentate gyrus and the effects of brief, high-frequency synaptic activation (8 firings at 400 Hz), which causes Ca^{2+} influx through NMDA channels into a modeled spine. Here we update our model of calcium's interaction with calmodulin to include the currently accepted kinetics of this interaction. The calmodulin concentration used in the simulations was 20 μM. The four Ca^{2+} binding sites on a calmodulin molecule were modeled using the different kinetic constants summarized in Cox et al. (In *Calcium and Calcium Binding Proteins*, 1988) for the situation in which binding proteins are present. In these conditions, binding of the third Ca^{2+} to a calmodulin molecule exhibits a very high cooperativity (ibid). Brief, high-frequency activation of a single synapse produces 2-5 μM of calmodulin saturated with four bound calcium ions. This concentration (according to the data of Meyer et al., *Science* 256:1199, 1992, and assuming that there is more calmodulin than CaMKinase II) is enough Ca_4Calmodulin to fully activate CaMKinase II. This may even be enough Ca_4Calmodulin to lock the CamKinase II holoenzyme in a permanent autophosphorylation mode. Thus, these data indicate that CaMKinase II activation alone will not be sufficient to produce associative LTP because, in vivo, single synapses are not sufficient for homosynaptic LTP.

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711.8

INVOLVEMENT OF Ni^{2+}-SENSITIVE CALCIUM CHANNELS IN HOMOSYNAPTIC LTD IN HIPPOCAMPUS L. K. Schexnayder*, B. R. Christie, and D. Johnston, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Recently we have shown that voltage-gated Ca^{2+} channels that are sensitive to Ni^{2+} (R- and T-type) are a major source of Ca^{2+}influx in the apical dendrites of CA1 pyramidal neurons (Christie et al., 1995, *J. Neurophysiol.*; Magee and Johnston, 1995, *Science*). In the present experiments we examined the role of these channels in homosynaptic LTD. Hippocampal slices were prepared from 2–4 week old rats using standard techniques and maintained in a submerged chamber. To assess basal synaptic strength, afferent fibers in the stratum radiatum were stimulated at 0.1 Hz. Initially we examined whether LTD could be induced at temperatures at which Ca^{2+} channel activity in dendrites has been examined. Slices maintained at 23°C exhibited a 19.9±7.4% (n=6) reduction in response size that lasted for at least 30 min following 1 Hz stimulation (900 pulses) and a 22.2±2.2% (n=5) reduction when 3 Hz was administered. No greater LTD was produced at 32°C, where the 1 and the 3 Hz produced −20.7±7.5% (n=7) and −21.7±12% (n=4) LTD, respectively. To evaluate the contribution of Ni^{2+}-sensitive channels to LTD induction, $NiCl_2$ was first added to the bathing medium for 25 min at one of three concentrations (25, 50 and 100 μM), and its effects on synaptic transmission were monitored for 30 min following washout. Both the 50 and the 100 μM concentrations produced a depression of synaptic strength when administered alone (50 μM: −21±4%, n=3; 100 μM: −20.1±5.2%, n=5). The 25 μM concentration, however, did not produce any discernable changes in synaptic strength (6.7±2.9%, n=5). Thus, in separate slices, we applied the 25 μM concentration during the administration of either the 1 or the 3 Hz stimuli and found that while a short-term depression was produced, Ni^{2+} blocked the expression of LTD (1Hz: 1.6±11.9%, n=6; 3Hz: 7.9±9.7%, n=6). (MH44754, MH48437, NS11535, NSERC103103).

711.9

FLUORESCENCE IMAGING OF INTRACELLULAR CALCIUM DURING THE INDUCTION AND EXPRESSION OF HOMOSYNAPTIC LTD IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. B. R. Christie*, J. Magee, and D. Johnston Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030

Hippocampal neurons exhibit various forms of synaptic plasticity, including long-term potentiation and long-term depression (LTD). The induction of LTD depends, among other things, upon a rise in postsynaptic $[Ca^{2+}]_i$. To investigate the changes in $[Ca^{2+}]_i$ during LTD, we have combined fura-2 imaging with whole-cell recordings of visually identified CA1 neurons.

Hippocampal slices (400 μm) were obtained from 2–4 week old rats using standard procedures and maintained at 32°C in a submerged chamber. To measure fluorescence changes in intracellular Ca^{2+}, fura-2 (125 μM) was included in the pipette solution, and relative changes in ΔF/F were measured using a cooled CCD camera in sequential frame transfer mode (25 ms frame interval). Whole-cell recordings were made from CA1 neurons located within 50 μm of the slice surface. Following a 10 min baseline period, cells were synaptically activated at 3 Hz and evoked EPSPs and changes in fluoresence were monitored. LTD, which lasted at least 30 min, only occured in those neurons in which sufficient stimulation was given to elicit postsynaptic action potentials during the 3 Hz stimuli (subthreshold: −7.1 ± 5.8%, n=3; suprathreshold: −30.5 ± 15%, n=4). During the application of the 3 Hz stimulation, only neurons in the suprathreshold group exhibited detectable changes in postsynaptic calcium levels (ΔF/F, soma: 7.4 ± 1.1%; 0–75 μm: 6.9 ± 1.5%; 75–150 μm 6.8 ± 4.1%; > 150 μm: 4.3 ± 2.9%). These data suggest that the Ca^{2+} entry during suprathreshold synaptic stimulation is involved in the induction and maintenance of LTD (MH44754, MH48437, NS11535, NSERC103103).

711.10

DYNAMICS OF CALCIUM AND CALCIUM-BINDING PROTEIN IN A SPINE : AN ANALYSIS BY MODELING AND SIMULATION K.Ichikawa*, H.Okamoto and I.Yamaguchi. Foundation Research Lab. Fuji Xerox Co., Ltd., 430 Sakai Nakaimachi Ashigarakamigun Kanagawa, 259-01 Japan

Increase in the intracellular calcium concentration($[Ca^{2+}]_i$) triggers both long-term potentiation(LTP) and depression(LTD) depending on stimulus intensity. Although mechanisms which underlie long-term synaptic modification are not yet elucidated, Ca^{2+} was found to play a key role. In the present study, we constructed a model for $[Ca^{2+}]_i$ and Ca^{2+}-binding proteins in a spine, and analyzed their dynamics.

The present model includes N-methyl-D-Aspartate(NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid(AMPA) receptor channels with desensitization, Ca^{2+} flux through NMDA receptor channels, Ca^{2+}-buffers, Ca^{2+}-binding protein, Ca^{2+} extrusion, and Ca^{2+} diffusion. Dynamics of intracellular $Ca^{2+}$and Ca^{2+}-binding protein for tetanic stimulation(100Hz-1s), low frequency stimulation(1Hz), and theta-burst stimulation were compared.

The increase in $[Ca^{2+}]_i$ was transient even synaptic stimulation continued. This is because AMPA and NMDA receptor channels are desensitized, and the inward flow of Ca^{2+} is reduced. Furthermore, the peak level of $[Ca^{2+}]_i$ was much higher in theta-burst stimulation than in tetanic (continuous) stimulation because of the slow recovery from desensitization. This result explains why theta-burst stimulation is effective for inducing LTP. In contrast, low frequency stimulation resulted in much smaller increase in $[Ca^{2+}]_i$ which would lead to LTD. The concentration of activated form of calmodulin increased far more drastically than the increase in $[Ca^{2+}]_i$ as stimulating intensity was increased. Therefore, the role of calmodulin is to amplify the Ca^{2+} signal and stabilize the differentiation between LTP and LTD.

711.11

A PARALLEL INCREASE OF NMDA AND NON-NMDA RECEPTOR-MEDIATED CURRENTS IN CALCIUM-INDUCED LTP IN CA1 PYRAMIDAL CELLS OF HAMSTER HIPPOCAMPAL SLICES. S.-N. Yang*, D. W. Robinson and J. M. Horowitz. Sec. of Neurobiol., Physiol., and Behav., Univ. of Calif., Davis, CA 95616

Previously we reported the establishment of Ca^{2+}-induced LTP in area CA1 of the hamster hippocampal slice (Soc. Neurosci. Abs., 20: 119.3, 119.4, 1994). Here, we further study the involvement of NMDA and non-NMDA receptor-mediated currents in Ca^{2+}-induced LTP. Whole-cell patch-clamp recording of excitatory postsynaptic currents (EPSCs) evoked by single shocks to Schaffer fibers, and action potentials induced by depolarizing current injections were made from CA1 pyramidal cells of hamster hippocampal slices (350μm). The average resting membrane potential was - 61.4 ± 0.8 mV (n= 19). In current clamp mode (Vm= - 65 mV), application of high Ca^{2+} (without stimulating Schaffer fibers) only evoked a short-term increase of the firing rate of action potentials (n= 4). In contrast, a long-term increase of the firing rate was seen if sufficient single shocks were applied during a high Ca^{2+} pulse (n= 2). In voltage clamp mode (Vm= - 60 mV), a high Ca^{2+} pulse induced a *parallel*, long-term increase of both NMDA and non-NMDA receptor-mediated currents of the dual component EPSC (n= 8). Both the scaled EPSCs (n= 3) and normalized amplitudes of the decay times of EPSCs prior to, and following Ca^{2+}-induced LTP showed a similar pattern (n= 3). In addition, following a stable Ca^{2+}-induced LTP, tetanization of Schaffer fibers does not provide any additional enhancement of either NMDA or non-NMDA receptor components (n= 5). These results suggest that Ca^{2+}-induced LTP shares basic common mechanisms with tetanically-induced LTP. Moreover, the maintenance of Ca^{2+}-induced LTP involves a similar increase in NMDA and non-NMDA receptor components and no change in decay times of EPSCs. [NASA Grant #NAG-2-788]

711.12

NITRIC OXIDE SYNTHASE INHIBITORS SELECTIVELY BLOCK LTP OF MINIMAL AMPLITUDE SYNAPTIC RESPONSES. P. F. Chapman*, Psychology Department, Neuroscience Program, University of Minnesota, Minneapolis, MN 55455

The hypothesis that nitric oxide (NO) contributes to LTP has received a considerable amount of experimental support. At the same time, many laboratories have presented evidence that inhibitors of nitric oxide synthase (NOS) fail to block LTP, or block only under restricted conditions. These results suggest that NO might regulate the threshold for LTP induction at CA1 synapses. I have tested an alternative hypothesis; that NO might be required for LTP induction only at a relatively small subset of CA1 afferent synapses. According to the hypothesis, these synapses would be the most readily excitable of a heterogeneous group of CA1 afferents, and thus the most likely to be activated by weak stimulation. Therefore, LTP at the low end of the input/output curve should be blocked by NOS inhibitors, even following strong tetanus.

I have used an experimental protocol in which I alternated "weak" stimulation (40 μsec pulses) with "strong" stimulation (100 μsec pulses). Experiments were conducted at 31.5°C, in slices taken from rats 15-150 days old. Stimulus intensities were adjusted so that strong stimulation elicited a response about 30% of the maximum evoked field EPSP slope; weak baseline stimulation evoked an EPSP with a slope between 0.05 and 0.09 mV/msec. In normal ACSF, ten theta bursts, given at "strong" intensity, produced LTP of responses evoked by both strong (133±6%) and weak stimuli (150±28%) measured 60 minutes after tetanus. In contrast, the same stimuli given in the presence of 100 μM NOArg produced normal LTP as measured with 100 μsec pulses (172±24%), but no LTP when measured with 40 μsec pulses (103±6%). Bath application of 100 μM NOArg had no effect on baseline responses measured with 40 μsec pulses (99±1% of pre-drug baseline), or on LTP induced before drug application (150±28% at 60 min, 144±14% at 120 min). The results indicate a role for NO in LTP induced under physiological conditions, and suggest the presence of important heterogeneities in SC/C afferents to CA1. Supported by NSF grant IBN-9410131

711.13

DIFFERENTIAL EFFECTS OF NITRIC OXIDE SYNTHASE INHIBITORS ON LTP AT BASAL AND APICAL SYNAPSES OF HIPPOCAMPAL CA1 NEURONS. J.E. Haley and D.V. Madison* Dept. of Molecular and Cellular Physiology. Stanford University School of Medicine, Stanford, CA 94305-5426.

Diffusible messengers such as nitric oxide have been proposed to act as intercellular signals in the production of long-term potentiation. Recent reports (O'Dell, et al. [1994], Science 265, 542-6; Wendland, et al.[1994], PNAS 91, 2151-5) illustrate that different forms of NOS are localized to the somata and apical dendrites, but appear to be absent from the basal dendrites of these neurons. Thus, LTP elicited in synapses terminating in the basal dendrites of CA1 pyramidal cells may not depend on NO, in contrast to apical synapses. We have tested this theory by comparing the effects of the NOS inhibitors on LTP elicited by electrical stimulation in stratum radiatum (s.r.; apical) and s.oriens (s.o.; basal). In control, tetanization (100 Hz/1 sec) in s.r. caused LTP of 140.3 +/- 6.8% of basal transmission; s. o. tetanization resulted in LTP of 156.9 +/- 13.3%. After application of L-NO-Arg (100 uM), identical tetanization in s.o. still produced normal LTP (156.4 +/1 17.9%) but following tetanization in s.r., LTP did not occur (111.1 +/- 9.3%). Utilizing intracellular recording, we attempted to see if we could detect this differential effect of NOS inhibitors when they were injected into single postsynaptic cells. In these experiments, LTP was induced by pairing postsynaptic depolarization (by current injection) with 1Hz stimulation delivered simultaneously to s.o. and s.r. In control , this resulted in LTP of 155.7 +/- 14.3% in s.r. and 138.1 +/- 13.6 % in s.o. After injection of L-NMMA (100 mM) into postsynaptic cells LTP was prevented in both pathways (s.r.: 113 +/- 9.06%; s.o.: 100.1 +/1 7.7%). Thus a differential effect on LTP was not seen when using intracellularly injected NOS inhibitor with pairing-induced LTP. Present results suggest that there may be a differential role for nitric oxide in apical vs basal dendritic synapses in area CA1 of hippocampus. However, not all experimental results are consistent with this hypothesis. This discrepancy is being investigated.

711.14

A POTENTIAL ROLE FOR PROTEIN MYRISTOYLATION AND AN ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) IN LONG-TERM POTENTIATION (LTP). D. B. Kantor, G. R. Sandoval and E.M. Schuman*. Division of Biology. California Institute of Technology 216-76, Pasadena, CA 91125.

Previous studies utilizing broad spectrum NOS inhibitors (which inhibit both eNOS and neuronal NOS, nNOS) have suggested that NO may participate in LTP. Physiological and immunohistochemical studies from nNOS and wild-type mice have suggested that eNOS, rather than nNOS, activity may produce NO during LTP. To test this suggestion we have utilized a putative nNOS specific inhibitor, 7-nitroindazole (7-NI). In two pathway experiments, we found that 7-NI-treated slices (100 μM) exhibited significant LTP that was of a similar magnitude to control slices [mean % of baseline: control: 131.8 +/- 4.3 (n = 8) 7-NI: 119.7 +/- 6.5 (n = 10)]. The most striking difference between eNOS and nNOS isoforms is the co-translational N-terminal myristoylation and consequent membrane localization of eNOS. Accordingly, we have begun to examine whether myristoylation of proteins is important for LTP production by utilizing hydroxy-myristic acid (HMA), an inhibitor of protein myristoylation. Slices pre-incubated in HMA for 18-24 hrs. in HMA (200 μM-1mM) failed to exhibit LTP wheras control slices incubated in solvent alone for the same time period exhibited significant potentiation [mean % of baseline: HMA: 109.7 +/- 7.8 (n = 10), control: 182.3 +/- 14.4 (n = 9)]. Post-hoc *in vitro* assays of myristoylation indicate that the HMA pre-treated slices (that failed to exhibit LTP) exhibited significantly reduced myristoylation of an exogenous myristoylation peptide when compared to controls [^{3}H-myristic acid incorporation (cpm) control: 4142.0 +/- 516.0, HMA: 271.0 +/- 28.0 (n = 5)]. We are currently developing an adenovirus construct to deliver recombinant cDNA encoding eNOS mutants which lack catalytic activity yet retain the N-terminal glycine required for myristoylation. In preliminary experiments we have infected slices with an Ad-*lacZ* construct and obtained β-gal expression within 4 - 18 hrs; these same slices exhibit normal LTP.

711.15

PHOTOLYTIC RELEASE OF NITRIC OXIDE BLOCKS INDUCTION OF LTP BY DEPRESSION OF NMDA RECEPTOR-MEDIATED TRANSMISSION IN THE CA1 REGION OF RAT HIPPOCAMPUS K.P.S.J. Murphy and T.V.P. Bliss* National Institute for Medical Research, Mill Hill, London NW7 1AA, U. K.

We have used flash photolysis of a caged form of nitric oxide ($K_2Ru(NO)Cl_5$) to rapidly deliver known concentrations of nitric oxide (NO) to area CA1 of hippocampal slices prepared from young Sprague-Dawley rats (70-100g) maintained in an interface chamber at 24°C. In previous experiments designed to test the retrograde messenger hypothesis for NO we were unable to detect NO-induced potentiation of synaptic efficacy (Murphy *et al*, (1994) *Neuropharmacology* **33**, 1375-85). We report here evidence that NO may instead serve a role in setting the threshold for the induction of LTP (cf. Izumi *et al*, (1992) *Science* **257**, 1273-76). Prior exposure of slices to photolytically released NO (1-4.5μM), ten minutes before application of a conditioning tetanus, blocked the induction of LTP in a concentration-dependent manner; a control pathway potentiated before application of NO was unaffected. The magnitude of LTP observed after exposure to NO, expressed as a percentage of LTP in the control pathway, was (mean±sem) 101.0±15.0, 41.0±9.9, 30.3±4.1 and -14.8±17.9 for 0μM, 1μM, 3μM and 4.5μM NO respectively (n=3 for each concentration). The mechanism by which NO regulates the induction of LTP is possibly by modulation of NMDA receptors. We have shown previously that photolytically released NO selectively depresses NMDA receptor-mediated transmission in a persistent and concentration-dependent manner. NO-induced reduction in the efficacy of the NMDA receptor could be sufficient to raise the threshold and/or block the induction of LTP.

NO may modulate NMDA receptors by redox modification. Such a reaction would be expected to produce a rapid alteration in NMDA receptor function. However, the time-course of NO-induced depression, following photolytic release of NO, is surprisingly slow. Exposure to 2.7μM NO induced a 63.6±12% (n=6 slices) depression of the initial slope, taking 104.3±14.3s to reach half-maximum depression, suggesting that redox modification is not the primary mechanism of NO-dependent depression.

711.16

MODULATION OF LONG-TERM POTENTIATION BY REACTIVE OXYGEN SPECIES. E. Klann*, E.D Roberson, and R.M. Mack. Dept. of Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260 and Div. of Neuroscience, Baylor College of Medicine, Houston, TX, 77030.

The induction of long-term potentiation (LTP) in hippocampal area CA1 is associated with an increase in cofactor-dependent protein kinase C (PKC) activity which is mediated by both nitric oxide (NO) and superoxide ($\bullet O_2^-$) (Soc. Neurosci. Abstr. 20:446). To test the hypothesis that PKC is directly activated by reactive oxygen species, we incubated purified PKC with SIN-1, a compound which produces NO and $\bullet O_2^-$. Incubation of PKC with 1 mM SIN-1 increased cofactor-dependent PKC activity (196 ± 14% of control, n=12). This increase was attenuated by superoxide dismutase (SOD, 25 μg/ml; 131 ± 13% of control, n=4) and 10 μM hemoglobin (132 ± 22% of control, n=4), suggesting that both NO and $\bullet O_2^-$ are necessary for the activation of PKC. Because NO reacts with $\bullet O_2^-$, ultimately forming hydroxyl radical ($\bullet$OH), we hypothesized PKC could be activated by $\bullet$OH. PKC was incubated with SIN-1 and 10 mM mannitol, an $\bullet$OH scavenger. Mannitol inhibited the SIN-1-induced increase in PKC activity (108 ± 9% of control, n=4), suggesting that $\bullet$OH directly activates PKC. Given these findings, we hypothesized that reactive oxygen species such as $\bullet O_2^-$ and $\bullet$OH could modulate induction of LTP. LTP-inducing stimuli was delivered to the Schaffer collateral input into area CA1 of rat hippocampal slices either in the presence or absence of SOD (121 U/ml). In control slices, LTP was observed in 8 of 9 experiments (initial slope of EPSP = 159 ± 9% of control). In contrast, slices incubated with SOD exhibited LTP in 1 of 9 experiments (initial slope of EPSP = 101 ± 8% of control). However, SOD converts $\bullet O_2^-$ to H_2O_2, which has been shown to inhibit LTP. Therefore, in addition to SOD, catalase (260 U/ml) was incubated with slices in order to remove H_2O_2. LTP was observed in only 5 of 15 slices incubated with SOD and catalase (initial slope of EPSP = 115 ± 6% of control), whereas LTP was observed in 12 of 13 control slices (initial slope of EPSP = 150 ± 4% of control). These data suggest that $\bullet O_2^-$ can modulate the induction of LTP. Further experiments are necessary to determine if $\bullet$OH can also modulate LTP and if PKC is a target for reactive oxygen species after LTP-inducing stimuli.

711.17

INDUCTION OF LONG-TERM POTENTIATION IN THE PRESENCE OF INHIBITORS OF NITRIC OXIDE IN RAT MOTOR CORTEX.

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Several studies have indicated that the induction of long-term potentiation (LTP) in the hippocampus may be dependent on the production of nitric oxide (NO). More recent studies have indicated that NO synthase inhibitors do not block LTP induction in all cases. To investigate whether NO is involved in the generation of activity-dependent synaptic plasticity in neocortex, we examined the role of NO in the production of LTP in rat motor cortex by administering extracellular NO synthase inhibitors. The results indicated that iontophoretic application of 50 mM N^G-nitro-L-arginine (NOARG) had no significant effects on the amplitudes of the population spike (PS) and EPSP. A similar result was observed when 40 mM hemoglobin was applied. Even at high concentrations of NOARG (80 mM) and hemoglobin (60 mM), the amplitudes of the PS and EPSP did not change significantly. LTP was induced by tetanic stimulation (50 Hz) in the presence of NOARG (50 to 80 mM) and hemoglobin (40 to 60 mM). Neither of these treatment significantly affected the induction of LTP.

The results presented here indicate that, in contrast to the hippocampus, NO does not play a major role in induction or maintenance of LTP in motor cortex. NO synthase inhibitors in the motor cortex have failed to reveal any significant effect on both evoked responses and the induction of LTP. These results suggest that NO is not necessary for LTP induction in motor cortex.

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711.18

β-AMYLOID ENHANCEMENT OF LTP AND NMDAR-MEDIATED TRANSMISSION IN RAT HIPPOCAMPUS

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β-Amyloid is released at central synapses and may have a role in modulating glutamatergic synaptic function. We have investigated this possibility in the rat dentate gyrus in vitro. Hippocampal slices were isolated and superfused using conventional techniques. Excitatory postsynaptic potentials and currents (epsps and epscs) evoked by electrical stimulation of the associational/commissural inputs to the dentate granule cells were recorded with extracellular and intracellular (whole cell patch clamp) electrodes respectively. β-Amyloid (1-40) was bath-applied (200nM) or applied intracellularly (100nM). NMDA receptor-mediated transmission was pharmacologically isolated using the AMPA receptor antagonist NBQX. Bath or intracellular application of β-amyloid resulted in a gradual enhancement of the NMDA receptor-mediated epsc which plateaued approx. 15 min later at a level averaging 140-160% of baseline ($P<0.05$). In those experiments where β-amyloid was washed off there was no reversal of the effect. Although there was no effect on basal AMPA receptor-mediated transmission after bath application of β-amyloid, high frequency stimulation-induced long-term potentiation (LTP) of the field epsp was enhanced by 30% ($P<0.05$). The enhancement of LTP of AMPA receptor-mediated transmission indicates that β-amyloid can facilitate synaptic plasticity. The long-lasting increase in basal NMDA receptor-mediated transmission is direct evidence that β-amyloid selectively interacts with this potentially neurotoxic target via a postsynaptic site.

LONG-TERM POTENTIATION: PHYSIOLOGY VI

712.1

LONG TERM POTENTIATION OF THE SPINAL CORD MOTONEURON DEMONSTRATED IN-VITRO IN THE SPINAL CORD OF NEONATAL RAT

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Long term potentiation (LTP) is a used dependent form of synaptic plasticity characterized by a long lasting enhanced of synaptic strength. In the hippocampus LTP appears to be the physiologic substrate of memory. The existence and possible role of LTP at the spinal motoneuron have not been fully demonstrated. We have used the immature spinal cord of rats (1-6 days old) in-vitro with recordings of ventral root field potentials (VRFPs) and simultaneous electrical stimulation of the dorsal nerve roots or corticospinal tract. Sequential short trains of tetanic stimulation at 100-150 Hz during 5 seconds induced long-lasting potentiation of VRFP amplitudes. This phenomenon was partially and reversibly abolished by perfusion of the preparation with 2-amino-5-phosphonovaleric acid (APV). Exposure of the spinal cord to the non-N-methyl-D-aspartate (non-NMDA) receptor blocker 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) completely and irreversibly abolished VRFPs. These findings suggest that LTP is present at the spinal motoneuron of neonatal rats. The partial obliteration of this phenomenon with APV suggest that LTP at the spinal motoneuron may involve the

712.2

SUSTAINED POSTSYNAPTIC POTENTIATION OF MINIATURE POSTSYNAPTIC POTENTIALS IN CULTURED RAT SYMPATHETIC NEURONS.

K.Kuba*, C.Liu and T.Shirasaki. Dept. of Physiology, Saga Medical School, Saga 849, Japan.

Nicotinic synapses formed between cultured neurons of rat superior cervical ganglia were studied with whole cell patch clamp and fura-2 fluorescence recording techniques. A rapid rise in extracellular K^+ to 20-40 mM by the jump of a laminar flow increased the frequency of miniature excitatory postsynaptic potentials (MEPSCs). The amplitude of MEPSC gradually increased to 120-200% during the course of a high K^+ treatment. On the other hand, the frequency fluctuated around a constant value occasionally with oscillation at a rate of several minutes. Furthermore, an accompanied rise in the intracellular Ca^{2+} of putative presynaptic neurons was unchanged or gradually reduced under the same condition. The inward current induced by acetylcholine after the end of a high K^+ application was also augmented, indicating the postsynaptic mechanism of MEPSC. The potentiation was seen for more than 20 min after the end of a high K^+ treatment and reversibly inhibited by phorbol ester (20 nM). Since the cytoplasm of the ganglion cell body was dialyzed through a patch pipette, the mechanism of potentiation may take place in the localized region of the subsynaptic membrane or may not involve a diffusible messenger.

712.3

SHORT- AND LONG-TERM PLASTICITY OF EXCITATORY SYNAPSES BETWEEN PAIRS OF CELLS IN HIPPOCAMPAL SLICE CULTURES.

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Pairs of monosynaptically coupled neurons were recorded in rat hippocampal slice cultures. When two action potentials were elicited in a single microelectrode impaled CA3 pyramidal cell, the amplitude of the first postsynaptic current (EPSC) in a whole-cell voltage-clamped CA3 or CA1 cell varied, and the amplitude of the second EPSC was inversely related to the amplitude of the first. Paired-pulse facilitation (PPF) was observed when the first EPSC was small and paired-pulse depression (PPD) when the first EPSC was large. The number of trials displaying PPD was greater when release probability was increased experimentally, and smaller when release probability was decreased. PPD was not affected by decreasing postsynaptic ion flux or receptor desensitization. We conclude that PPD results from a decrease in quantal content, perhaps due to short-term depletion of readily releasable vesicles.

Long-term potentiation (LTP) and depression (LTD) in the hippocampus may be induced alternatively with synaptic responses resulting from the activation of many axons with extracellular stimulation. It is, however, still unknown whether unitary synapses may undergo both LTP and LTD. Pairs of pyramidal neurons in areas CA3 and CA1 of rat hippocampal slice cultures were recorded with sharp microelectrodes. Presynaptic high frequency tetani induced with 2-5 bursts of action potentials at 50 Hz in the presynaptic cell, were ineffective in inducing LTP. LTP could, however, be induced with synchronous pairing of single presynaptic action potentials and postsynaptic depolarizing pulses. This potentiation was reversed by induction of LTD using either low frequency tetani (3 Hz) or asynchronous pairing. These results demonstrate that the same unitary synapses may undergo mutually reversible long-term potentiation and depression.

712.4

THE DEVELOPMENT OF DENDRITIC SPINES IN HIPPOCAMPAL ORGANOTYPIC CULTURES.

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We have investigated developmental changes in the size and density of dendritic spines on CA1 pyramidal cells in organotypic cultures using confocal microscopy.

Cultures were prepared from three day old Wistar rats using a method adapted from Stoppini *et al. (J. Neurosci. Meth. 37, 173, 1991)*. At 5-7, or 21-26 days, CA1 pyramidal neurones were iontophoretically injected with lucifer yellow dye, and imaged with a Biorad MRC 1000 confocal microscope. The architecture of CA1 cells from a mature organotypic culture (> 3wks) is comparable to that seen in CA1 neurones in acute slices from 3-4 week old rats. The stereologically uncorrected mean interval between spines on secondary apical dendrites was 1.4 ± 0.17 μm (n=40), and on basal dendrites was 1.7 ± 0.13 μm (n=128); the mean observed spine length was 1.12 ± 0.04 μm (n=46) for apical dendrites and 0.80 ± 0.02 μm (n=146) for basal dendrites. These values are similar to those reported in acute slices for Golgi stained CA1 cells. In contrast, spine density from immature cultures (< 1wk) was significantly less (mean density: 2.07 ± 0.26 μm (n=33) for apical dendrites, ($p < 0.01$), and 2.35 ± 0.37 μm (n=39) for basal dendrites, ($p < 0.001$). The length of spines however, was not significantly different from that observed in mature slices (mean length = 1.01 ± 0.06 μM, (n=37) for apical spines (N.S.), and 1.10 ± 0.07 μM (n=48) for basal spines (N.S.)).

These results indicate that CA1 neurones in organotypic hippocampal cultures systematically add spines during development, so that after three weeks a density is achieved which is comparable to that observed in acute slices, despite the fact that there are fewer CA3 projection cells in organotypic cultures than in the intact animal from which acute slices are cut. This implies either that the number of CA3 cells projecting to a given CA1 cell is greater in organotypic cultures than in acutely prepared hippocampal slices, or that each CA3 cell makes a greater number of contacts per target cell. The physiological observations of Debanne *et al. (J. Neurophys. 73, 1282, 1995)* indicate that the former is primarily the case.

712.5

DENDRITIC SPINES IN HIPPOCAMPAL NEURONS: CORRELATING STRUCTURE AND FUNCTION. C. Collin*[1], K. Miyaguchi[2], and M. Segal[3]. [1]LAS, [2]LN, NINDS, NIH, Bethesda MD, 20892 Dept. of Neurobiology, The Weizmann Institute, Rehovot 76100, Israel.

Dendritic spines have been proposed to constitute the locus of long-term memory storage in central neurons, yet little is known about the biophysical and morphological mechanisms underlying their role in memory. We studied the effects of chronic exposure to drugs which affect synaptic transmission on spine morphology and ability of slices to express long term potentiation (LTP) of population responses to afferent stimulation in area CA1 of rat hippocampal slices maintained in culture conditions for 1-5 weeks. Blockers of excitatory or inhibitory neurotransmission were added to some cultures. Extracellular field EPSP's (fEPSP) were recorded in field CA1 in response to stimulation of CA3 neurons. Individual cells were labelled with lucifer yellow, and were subsequently visualized in a confocal laser scanning microscope. Within 10 days in culture, CA1 dendrites had large filopodia-like structures and almost no spines. fEPSP amplitude was 3.5±1.1mV and tetanic stimulation (100Hz, 1 sec) caused no LTP. At 3 weeks, spines were present in large heterogeneity (mean density=0.81 spines/μm of dendrite), fEPSP amplitude was 5.6 ± 0.9 mV, and tetanic stimulation caused LTP in 63% of the slices. At 5 weeks, spine density was higher, but no significant increase in the fEPSP amplitude or ability to produce LTP was observed. Slices grown in the presence of picrotoxin did not show changes in spine density at 3 weeks, but the fEPSP amplitude was significantly smaller than controls (1.9 ± 0.6 mV). Tetanic stimulation failed to induce LTP. Spine density was reduced in slices grown in the presence of the NMDA antagonist APV, fEPSP amplitude was same as controls, but LTP was induced in 100% of the slices. The data suggest that the maturation of dendritic spines in hippocampal neurons is age-and activity-dependent. The ability to express LTP is correlated with the appearance of spines, but not with variations in spine morphologies.

712.6

QUANTIFICATION OF DENDRITIC SPINES AND SYNAPSES IN RAT HIPPOCAMPAL SLICES MAINTAINED FOR VARIABLE TIMES *IN VITRO*. K.E. Sorra*, B.A. Allwardt, and K.M. Harris. Dept. Neurol. & Prog. Neurosci., Children's Hospital and Harvard Medical School, Boston, MA 02115.

Hippocampal slices are widely used as a model system in which to study the cellular mechanisms of synaptic transmission and plasticity. As part of an ongoing study of the structural basis of synaptic plasticity (LTP), slices were evaluated electrophysiologically and the number and structure of dendritic spines were compared among slices that were maintained for short (1.75-2.5 hrs) or long (6-13 hrs) time periods *in vitro* and with previous *in vivo* values (Harris et al., 1992, J. Neurosci. 12:2685-2705). The unbiased series sample analysis was used to compute synapse densities and identify dendritic spine shapes. In the *in vivo* adult hippocampus, thin spines predominate whereas in the immature hippocampus stubby, mushroom and thin spines occur in roughly equal numbers. In adult slices overall synapse densities were equal at both *in vitro* time periods, but greater than those observed *in vivo*. This apparent increase occurred among the stubby and mushroom spines and did not affect the frequency of thin spines which still predominated across all conditions. These observations suggest that the *in vitro* condition *per se* could induce or alter dendritic spines; however the structural modifications appeared by 2 hrs and were relatively stable for up to 13 hrs *in vitro*. This stability suggests that the slice system is appropriate for ascertaining whether changes in synaptic efficacy, such as LTP, specifically alter spine and synapse structure. Preliminary observations from immature hippocampal slices suggest that all classes of spines are present and quantitative analysis will determine whether stable modifications also occur in immature spines maintained *in vitro*. Supported by NSERC fellowship (KES), NRSA fellowship #F31MH10916 (BAA), NS21184, and the MR center grant P30-HD18655.

712.7

LONG-LASTING POTENTIATION IN CULTURED HIPPOCAMPAL PYRAMIDAL CELLS REQUIRES NMDA RECEPTOR ACTIVATION, Ca^{2+} AND NO SYNTHASE IN THE POSTSYNAPTIC CELL, AND G-KINASE. O. Arancio* and R. D. Hawkins. Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032.

We have begun to compare the properties of long-lasting potentiation in dissociated hippocampal cell culture with LTP in slices. Pairs of pyramidal-shaped neurons were maintained throughout the experiment under whole-cell voltage clamp. Three high-frequency trains of depolarization (50 Hz, 2 sec) of the presynaptic neuron in O Mg^{2+} saline produced immediate potentiation of the EPSC that lasted until the end of the experiment (average = 35% increase, $p < .01$, $n = 11$). There was no potentiation in experiments in which the bath solution contained the NMDA receptor blocker APV (50 μM) or in which the tetanic stimulation was delivered in normal Mg^{2+} saline ($n = 12$). We were able to produce long-lasting potentiation in normal Mg^{2+}, however, by pairing low-frequency stimulation of the presynaptic neuron (1 Hz for 30 sec) with depolarization of the postsynaptic neuron to O mV (average = 48% increase, $p < .01$, $n = 8$). Potentiation by tetanic stimulation was blocked by injection of the Ca^{2+} chelator BAPTA (20 mM) into the postsynaptic neuron ($n = 7$). Potentiation was also blocked by injection of the NO synthase inhibitor N^{ω}-methyl-L-arginine (0.5 μM) into the postsynaptic ($n = 9$) but not the presynaptic ($n = 8$) neuron. Conversely, long-lasting potentiation could be produced by bath application of NO (10 nM) paired with weak tetanic stimulation (50 Hz, 0.5 sec) of the presynaptic neuron (average = 37% increase, $p < .02$, $n = 10$). Finally, potentiation by either tetanic stimulation ($n = 7$) or low-frequency stimulation paired with postsynaptic depolarization ($n = 8$) was blocked by bath application of the G-kinase inhibitor Rp-8-Br-cGMPS (100 μM).

712.8

PRESYNAPTIC INDUCTION OF MOSSY FIBER LONG-TERM POTENTIATION IN DISSOCIATED CELL CULTURE. J. C. López-Garcia* and D. Baranes. HHMI, Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032.

It is widely accepted that long-term potentiation (LTP) in the Schaffer collaterals of the hippocampus requires postsynaptic activation of the NMDA subtype of glutamate receptors. In contrast, LTP in the mossy fiber pathway (MF) is NMDA receptor-independent. Attempts to identify its loci of induction and expression have yielded contradictory results due in part to the complex circuitry of the hippocampal slice. In the present work, we addressed this question using a simplified MF system composed of individual dentate gyrus granule cells and CA3 pyramidal neurons in dissociated cell culture. After 12-14 days *in vitro*, the two cell types present in the culture established glutamatergic synaptic connections that showed striking similarities with the intact MF: they had both non-NMDA and NMDA receptors and were modulated by the opioid peptide dynorphin A. Furthermore, this connections displayed NMDA-independent LTP. High-frequency stimulation of a single granule cell in the presence of NMDA receptor blockers produced a long-lasting increase in the amplitude of the excitatory postsynaptic currents recorded from a pyramidal neuron. This effect was not seen when the presynaptic cell was also pyramidal. A coefficient of variation analysis of the responses before and after the induction of LTP showed that their variance increased in direct proportion to the change of their mean amplitude, indicating a presynaptic mechanism for the induction of this form of synaptic plasticity. These experiments constitute the first demonstration of NMDA-independent LTP in cell culture and provide strong evidence of the presynaptic nature of mossy fiber LTP.

712.9

FORSKOLIN INDUCES THE EXPRESSION OF TISSUE-PLASMINOGEN ACTIVATOR (tPA) mRNA AND THE RELEASE OF ITS PRODUCT FROM PRESYNAPTIC SITES OF DISSOCIATED HIPPOCAMPAL CELLS IN CULTURE. D. Baranes*, K. Martin, D. Lederfein. HHMI, Ctr. Neurobiol. & Behav., Columbia Univ., NY, NY 10032.

Tissue plasminogen activator (tPA) has been implicated in a number of cellular processes in the nervous system such as axonal elongation and cell migration. Qian et al. (Nature 361:453, 1993) have demonstrated that long-term potentiation (LTP) increases tPA mRNA expression in the dentate gyrus. We have found that forskolin, an adenylyl cyclase activator, which produces LTP in the mossy fiber pathway connecting hippocampal granule cells to CA3 pyramidal cells (Huang et al., Cell 79:69, 1994), induces a five-fold increase in tPA mRNA levels in cultured neurons of the hippocampal mossy fiber pathway. Forskolin also induces a Ca^{2+}-dependent release of tPA beginning more than 1 min after application and reaching a peak 5 min after application.

Immunocytochemical analysis revealed that tPA is enriched at points where axons contact dendrites and is extensively colocalized with synaptophysin, a marker of presynaptic terminals. Of the positive sites, 73.5 ± 9.9% colocalized with synaptophysin, 26.5 ± 9.9% included tPA but no synaptophysin immunoreactivity, and 36.4 ± 11.3% of synaptophysin positive sites did not contain tPA immunoreactivity. In addition, western blot analysis of rat brain extracts revealed that tPA is present in synaptosomal fractions. Thus, these results suggest that tPA is present in presynaptic terminals and that its activity is increased during synaptic plasticity, both by transcriptional induction and by release of its product.

712.10

TISSUE-PLASMINOGEN ACTIVATOR PLAYS A CRITICAL ROLE IN THE LATE STAGE OF HIPPOCAMPAL LTP. Y.-Y.Huang*, T. Abel, and E. R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, NY, NY 10032.

Long-term synaptic potentiation in the hippocampus can be divided into an early phase (E-LTP) and a late phase (L-LTP). L-LTP requires new protein and RNA synthesis. However, little is known about the identity of the proteins involved. Tissue-plasminogen activator (tPA) is an extracellular serine protease that is induced by neural activity and is correlated with morphological differentiation of neurons. We found that the brief application of tPA to hippocampal slices induces a slowly-developing and long-lasting (> 5 hr) synaptic potentiation in both the Schaffer collateral-CA1 pathway and mossy fiber-CA3 pathways. In each of these pathways, the tPA-induced potentiation is occluded by potentiation induced by a cAMP analog. In addition, a tPA inhibitor selectively blocks the late phase of LTP induced by three trains of tetanus but not the early phase of LTP induced by one train in both the CA1 and mossy fiber pathways. Moreover, the tPA inhibitor also blocks the cAMP analog-induced potentiation. These results indicate that tPA may play an important role in the late phase of LTP in both the NMDA-dependent Schaffer collateral-CA1 and the NMDA-independent mossy fiber-CA3 pathways. tPA appears to be one of the proteins by which cAMP mediates the late phase of LTP.

712.11

Mechanism of LTP at parallel fiber synapses of the cerebellum.
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It has recently been shown in hippocampus that LTP at mossy fiber (MF) synapses is induced and expressed presynaptically and may be mediated by cyclic AMP (Weisskopf et al, 1994). The Ca entry during a tetanus is proposed to activate a Ca sensitive adenylyl cyclase (ACI), leading to an increase in cAMP. This increase, via PKA causes a persistent enhancement in evoked glutamate release. Because ACI is highly expressed in parallel fibers (PF) we examined whether a similar form of LTP occurs at parallel fiber-Purkinje cell synapses in rat cerebellar slices. Extracellular recording techniques enabled us to monitor simultaneously both the fiber volley and postsynaptic response. Low frequency stimulation (LFS, 4Hz for 20 sec) caused an increase in the field amplitude of 30% that lasted at least 1 hour. In most cases the fiber volley was only modified transiently after LFS, but in some cases was enhanced over a prolonged period of time. PF LTP was associated with a decrease in paired pulse facilitation (PPF) and application of the glutamate receptor antagonist kynurenate (10mM) during LFS of PF did not prevent the induction of LTP. These results indicate that PF LTP is presynaptic in induction and expression, as previously suggested in cultured cerebellar neurons. Furthermore, a short term application of the adenylyl cyclase activator forskolin caused an enhancement of PF synaptic responses that lasted at least 1 hour and induced a decrease in PPF. These experiments strongly suggest that common mechanisms are involved in PF and MF LTP. *Supported by the NIH.*

712.12

LONG-TERM POTENTIATION OF NMDA-MEDIATED RESPONSES AT THE PRIMARY OLFACTORY SYNAPSE.
M. Ennis*, L.A. Zimmer, & M.T. Shipley. Dept. Anat., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Mitral cells (MCs) of the main olfactory bulb receive direct excitatory synapses from primary olfactory nerve (ON) terminals. Our recent studies demonstrated that glutamate released from rat ON terminals activates MCs via non-NMDA (i.e., AMPA/kainate) and NMDA receptors; AMPA/kainate receptors mediate rapid, brief excitation of MCs while NMDA receptors mediate a delayed excitation lasting several hundred msec. In cortical circuits, brief epochs of high frequency stimulation potentiate responses at NMDA and non-NMDA receptors, i.e., LTP. The goal of this study was to determine if excitatory responses at the ON->MC synapse undergo frequency dependent LTP.

Extracellular recordings were obtained from rat MCs in 400 μm-thick horizontal sections and a stimulation electrode was placed on the ON layer (ONL). A brief, low intensity (4-40 μA) train of impulses (10 sets of 4 pulses @ 100 Hz; each set separated by 200 msec) was applied to the ONL. This stimulation pattern corresponds to 2 sec of investigative sniffing typical for rats responding to novel odors or exploring the environment.

A single 2 sec train produced immediate, dramatic increases in NMDA excitatory responses in 13/15 cells, ranging from 126% - 345% of control (mean = 189 ± 17%; $p < 0.001$). By contrast, AMPA/KA responses were not altered (mean = 94 ± 4%; $p > 0.5$). Potentiation was long-lasting (50 min), comparable to LTP in hippocampus and piriform cortex. The NMDA receptor antagonist AP5 (50-100 μM) prevented the induction of LTP; LTP was obtained after AP5 washout. AP5 also reversed LTP following its induction.

These results demonstrate that ON—>MC synaptic transmission exhibits use-dependent plasticity *in vitro*. A two sec burst (corresponding to 10 sniffs) of ON activity induces specific LTP of NMDA mediated excitation of MCs. LTP at the ON—>MC synapse discloses a novel mechanism for rapid amplification of MC responses to sensory inputs that may function in rapid memory formation for specific odors. Our results suggest that stimuli evoking bursts of ON activity may potently modify the synaptic excitability of MCs *in vivo*. Future experiments will test this prediction. *Support: NIH DC00347, NS29218 and NS29635.*

712.13

SPATIAL LOCALIZATION OF CURRENT SINKS IN THE SUPERFICIAL LAYERS OF THE ENTORHINAL CORTEX FOLLOWING PYRIFORM CORTEX STIMULATION IN THE RAT.
C.A.Chapman* and R.J.Racine. Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8S-4K1.

Field potentials in rat entorhinal cortex are potentiated for very long periods following strong stimulation of the pyriform cortex (SN Abstr 20:585.2), but the synaptic populations underlying these effects have not been spatially localized. Biphasic test pulses (225 to 650μA) were delivered to the pyriform cortex (AP-3.6, L6.5, V8.5-10mm) of urethane anesthetized (1.5g/kg) rats. A stainless steel (50μm tip) or tungsten recording electrode was advanced on the sagittal plane, 40° off horizontal and perpendicular to entorhinal cortex lamina, and aimed at a point 5.2mm lateral to the midline, and 2.6mm dorsal and 0.2mm anterior to the interaural line. Averages of 10 evoked field potentials were recorded at 50μm intervals at depths between 0.0 and 2.0mm, and electrode positions were verified using the Prussian Blue reaction, or with small lesions. The 2nd spatial derivative of smoothed depth profiles quantified the current source density in relative units. The major field potential component was spatially diffuse and approximately -0.55mV in amplitude at depths near 250μm with a ≈10ms onset latency and ≈18ms latency to peak. It corresponded to a current sink in layers II and III which peaked at depths between 250 and 350μm. A smaller, more prolonged sink with a ≈33ms onset latency was also observed in the deep layers. These results indicate that pyriform cortex efferents which support LTP terminate in the superficial layers of the rat entorhinal cortex.

712.14

LOW-FREQUENCY STIMULATION DEPOTENTIATES LTP IN RAT PIRIFORM CORTEX.
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Long-term potentiation (LTP) is a form of synaptic plasticity which is thought to be a mechanism underlying memory formation at mammalian central synapses. Recently, LTP has been under intense scrutiny with regard to a modulatory action of low-frequency stimulation, which itself can lead to long-term depression (LTD), a decrease in synaptic efficacy observed in the cerebellum and hippocampus. Here we report that in the rat piriform cortex low-frequency stimulation delivered to an already potentiated pathway depotentiates the test response to the control level.

Brain slices (450 μm thick) of the piriform cortex were prepared from young adult male rats (150 - 200 g) in Na-free Krebs-Ringer solution (in mM: NaCl 125.5; KCl 3.5; $CaCl_2$ 2.4; $MgSO_4$ 1.3; KH_2PO_4 1.2; $NaHCO_3$ 26; glucose 10; NaCl was replaced by 213.3 mM sucrose) and allowed to recover in standard Ringer solution (pH 7.4 after equilibration with 95% O_2/ 5% CO_2) for 1 1/2 h. Slices were transferred to the recording chamber (submerged type) and continuously perfused at a rate of 3 ml/min. Test stimuli (50 μs; 10-40 V; 0.1 Hz) were delivered to the lateral olfactory tract (LOT) and the dendritic field EPSP's were recorded with a large (30 μm) extracellular electrode. LTP was induced by either a single tetanus (1 s at 100 Hz) or a θ-burst paradigm (10 bursts consisting of 5 pulses at 100 Hz at an interval of 200 ms). After the potentiated response was monitored for 30 min (114.2 ± 2 %, mean ± S.E.; n=4) low frequency stimulation (900 pulses delivered at 2 Hz) caused a significant reduction of the EPSP's to the control level (98.5 ± 3%; $p < 0.05$). The depotentiation lasted for the remainder of the recording (usually 30 min) and in one attempt could be reversed to the initial LTP level by an additional θ- burst.

This suggests that a potentiated synapse may still undergo plastic changes contingent upon subsequent synaptic activity. Hence, a switch may exist between the potentiated and the depotentiated state, giving a synapse additional bandwith to respond to a particular stimulus. Supported by NS 23807-10 (DOC).

712.15

LONG-TERM MODIFICATIONS OF SYNAPTIC TRANSMISSION IN RAT VISUAL CORTEX INDUCED BY INTRACELLULAR TETANIZATION
M.Volgushev, L.L.Voronin, M.Chistiakova, C.Hansel, W.Singer*
Max-Planck-Institute for Brain Research, 60528 Frankfurt/M, Germany.

We examined the possibility to induce long-term changes in synaptic transmission in the visual cortex by applying bursts of intracellular pulses (intracellular tetanization, IT) to the postsynaptic cell, without concomitant presynaptic stimulation. Postsynaptic potentials evoked from two stimulation sites in layers II and IV were recorded from layer II-III cells with sharp or whole cell electrodes. IT led to marked changes in synaptic transmission in the majority of cells. Fura-2 based Ca^{2+} measurements showed that IT produced a substantial increase in intracellular $[Ca^{2+}]$, comparable in magnitude to increases evoked by strong afferent tetanization. Inputs from layer IV were more often potentiated while layer II inputs were more often depressed. This difference is probably due to the different localization of the respective afferents at the dendritic tree. Ca^{2+} imaging showed that stimulation in layer IV activates inputs to the proximal parts of the apical dendrites, while stimulation in layer II activates more distally located inputs. To evaluate the involvement of presynaptic mechanisms in the lasting changes of synaptic transmission induced by IT, we analysed paired-pulse interactions before and after potentiation. Paired-pulse facilitation (PPF) usually decreased after induction of long-term potentiation (LTP) and the magnitude of the decrease correlated positively with LTP magnitude and with the pretetanic PPF ratio.

Our data show that manipulations of the postsynaptic cell, which lead to a significant rise of intracellular $[Ca^{2+}]$ are capable to produce long-term changes in synaptic transmission in the visual cortex. Changes in PPF ratio after LTP induction suggest that presynaptic mechanisms are involved in the maintenance of synaptic modifications induced by IT.

712.16

TRANSCALLOSAL INDUCTION OF LONG-TERM POTENTIATION AND DEPRESSION IN THE SOMATOSENSORY AND VISUAL CORTICES IN THE AWAKE RAT.
D.J. Froc and R.J. Racine*. Dept. of Psychology, McMaster University, Hamilton, Ontario., L8S 4K1.

We have recently found that long-term potentiation can be induced in the neocortex of chronically prepared rats, but only if the stimulation is applied over multiple sessions. These experiments were done with both stimulation and recording electrodes in the same hemisphere. The present experiments were designed to determine whether neocortical LTP could be induced if the stimulation and recording electrodes were in homologous sites in opposing hemispheres. We also tested the effects of low frequency stimulation trains. These have been reported to produce *depression* effects in the hippocampus and visual cortex. Thirty, 24 msec, 300 Hz trains were applied to the neocortex each day for 10 days. Stimulation intensity was set at 1259 μA. In one group, the trains were applied to the somatomotor cortex and in another group, the trains were applied to the visual cortex. Control groups were also run for each site. Before and after the application of the potentiating trains, pulses were applied to the stimulation site and the responses evoked in the contralateral cortex were recorded and analyzed. One week following the induction of LTP, 1 Hz trains were applied to the stimulation site for 15 min after which the test pulse runs were resumed. We found that the baseline response amplitudes were larger in anterior sites, but the LTP effects were similar in both sites. The potentiation had shown little decay 1 week following the completion of the train stimulation. At that point, the low frequency trains were applied in half the animals from each group. A transient depression effect was found following low frequency stimulation of the anterior site. These responses had returned to their potentiated levels by 24 hours. The depression effect following stimulation of visual cortex was still expressed 7 days post-treatment. The control animals showed only minor depression effects in their baseline responses.

712.17

NEOCORTICAL LONG-TERM POTENTIATION IN THE CHRONIC PREPARATION: INDUCTION AND DECAY PARAMETERS. C. Trepel* and R. J. Racine. Dept. of Psychology, McMaster Univ., Hamilton, Ontario, Canada, L8S-4K1.

The belief that long-term memories are stored in the neocortex suffers from the fact that neocortical LTP (the leading physiological memory model) has, until recently, been successfully induced only in acute and slice preparations. We have found, however, that LTP can be induced in the neocortex of chronically prepared rats if multiple stimulation sessions are used. Here we report the results of experiments designed to provide a more complete description of the induction requirements and the time course for neocortical LTP. Stimulating and recording electrodes were implanted into the corpus callosum and anterior neocortex (frontal area 1), respectively. Daily stimulation consisted of varying numbers of 8-pulse, 24 ms trains (pulse frequency: 300 Hz; train frequency: 0.01 Hz). Four groups of animals received one of either 10 trains/day for 10 days (Group 1), 60 trains/day for 10 days (Group 2), 10 trains/day for 25 days (Group 3) or 60 trains/day for 25 days (Group 4). Compared to baseline measures, all animals showed developing potentiation after three sets of trains. This was evidenced as an initial increase and subsequent decrease in the amplitude of the early component (latency-to peak range: 4.5-6.8 ms) of the EPSP, an increase in the amplitude and number of population spikes, and an increase in the amplitude of the late component (latency-to-peak range: 15.9-29.5 ms). In many animals this late component is clearly seen only following potentiation. These potentiation measures increased over the course of trains in all animals. Animals receiving 60 trains/day required fewer trains to reach asymptotic levels of potentiation of the late component and displayed both a larger increase and subsequent decrease of the early component. LTP persistence data are currently being collected and preliminary observations indicate that the groups receiving 60 trains/day have the longest LTP decay time constants. These chronic data provide much needed evidence that a presupposed center of long-term memory is capable of supporting LTP effects that can last for weeks or months.

712.18

ANALYSIS OF LONG TERM POTENTIATION IN THE CHICK IMHV REGION. E. Shimabayashi, K. Kiyosue, M. Kasai*, T. Taguchi†. Dept. of Biophys. Engineer., Fac., of Engineer. Sci., Osaka Univ., Toyonaka 560, Japan, †Osaka Nat'l. Res. Inst., Ikeda 563, Japan

The intermediate and medial part of hyperstriatum ventral (IMHV) is critical part of chick forebrain to establsh imprinting and many types of learning. Although many electrophysiological studies have been performed by Bradley et al., analysis with whole cell recording has not been carried out, which is necessary to understand precise mechanizms of synaptic plasticity in this area. Using conventional patch clamp technique, we have analyzed long term potentiation (LTP) in IMHV region, where E.P.S.C.s were evoked by local stimulus near the postsynaptic cell. The stimulus of 5 Hz for 1 min paired with postsynaptic depolarization was used to induce the potentiation. The potentiations were sustained for 80 min at most. The coefficient of variation of peak amplitude of E.P.S.C. did not change so much after the induction of potentiation. If the probabilistic transmitter release is governed by binomial process, this dose not indicate increase of transmitter release probability but increase of postsynaptic efficacy. Further, the ratio of AMPA/kinate type glutamate receptor-mediated currents to NMDA type glutamate receptor-mediated currents changed after conditioning stimuli. These results indicate that postsynaptic change is included in the mechanism for LTP expression in this area.

712.19

SYNAPTIC PLASTICITY IN THE DIRECT FEEDBACK PATHWAY TO THE ELECTROSENSORY LATERAL LINE LOBE OF *A. LEPTORRHYN-CHUS*. D. Wang, L. Maler and P. Fortier*, Dept.of Anatomy and Neurobiology, University of Ottawa. Ottawa, Ontario Canada. K1H 8M5

Electroreceptor afferents terminate in a laminated rhombencephalic structure: the electrosensory lateral line lobe (ELL). In addition to its peripheral input, the ELL also receives feedback input: feedback reaches the molecular layer of the ELL via a direct feedback projection, the stratum fibrosum (SF) which terminates in the ventral molecular layer (VML). Earlier studies have revealed that the feedback projections were glutaminergic and associated with NMDA receptors; further the input to VML has been hypothesized to be involved in attentional processes. We therefore used an *in vitro* preparation of the ELL (brain slice) to investigate possible synaptic plasticity in the VML.

We stimulated the SF at frequencies mimicking those which occur during natural activation of this pathway in vivo (20-300 Hz); optimal tetanic stimulation (100 Hz for 100 ms repeated 3 times at 1 Hz) produced a 30-40% posttetanic potentiation (PTP) of the field EPSP at 5 s; this PTP decayed to baseline after 1-2 minutes. Intracellular recording confirmed the potentiation of the EPSP and demonstrated that it was maximal (about 80%) 1 s post-tetannus. Stimulation in the presence of manganese prevented PTP, suggesting that it represents synaptic potentiation and not a lowered threshold of the SF. We did not find any evidence of LTP, even when tetanus was delivered in the presence of bicuculline.

We conclude that transient memories of sensory input may be important in attentional modulation of sensory input, and that NMDA receptors need not always be involved in traditional LTP.

712.20

HUMAN CORTEX SHOWS ROBUST NMDA DEPENDENT LONG TERM POTENTIATION. S.H. Lee*, W. Chen, , K. Kato, D.D. Spencer, G.M. Shepherd, and A. Williamson. Sections of Neurosurgery and Neurobiology, Yale University School of Medicine, New Haven, CT 06520

LTP has been extensively studied in animal models but not in the human cortex. Here we report the induction of LTP in human neocortex, and its dependence on NMDA receptors. The tissue was obtained from temporal lobectomies for control of intractable epilepsy. We harvested pathologically and electrographically normal cortical specimens from four patients. 500 µm slices were prepared and maintained in an interface chamber. EPSPs were measured in layer II/III following stimulation in layer IV. LTP was induced by giving five trains of tetanic stimulation (100 Hz for 1 sec each) at 30 sec intervals. The EPSP amplitudes were monitored for 20 min pre-tetanus and for 60 min following the stimuli.

In total, we performed 28 trials using these parameters in 17 slices. LTP was produced in 11 of 18 trials performed in normal ACSF. Averaged data from these 11 trials showed a 23.6 ± 8.59% potentiation from pre-tetanus EPSP amplitude 10 min. after the tetanus, and a 34.5 ± 6.64% potentiation 55 min after the tetanus. The remaining 7 of 18 trials showed no potentiation of the EPSP amplitude. Activity-dependent synaptic plasticity was modulated but not eliminated by NMDA antagonist, APV. In 10 trials following the bath application of 50 µM APV, LTP was only seen in 2 of 10 trials. Chi Square analysis showed a highly significant effect of APV in decreasing LTP induction ($P<0.0001$).

Our study shows that LTP can be induced in adult human neocortical slices without the application of bicuculline. The incidence of LTP (61%) is comparable to that seen in the sensory cortex of young rats. We found that the incidence of LTP is significantly decreased by the application of NMDA receptor antagonist APV, suggesting that NMDA receptors are also involved in the plasticity seen in human neocortex.

LIGAND-GATED ION CHANNELS III

713.1

PROTEIN KINASE A OR PROTEIN KINASE C DOES NOT APPEAR TO BE INVOLVED IN THE INHIBITION OF ATP-ACTIVATED CURRENT BY ETHANOL IN SENSORY NEURONS. Chaoying Li*, Jin Zhai and Forrest F. Weight. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse & Alcoholism, National Institutes of Health, Bethesda, MD 20892-8205.

We have previously demonstrated that ethanol, in pharmacological concentrations, produced concentration-dependent inhibition of ATP-activated inward current in freshly isolated bullfrog dorsal root ganglion neurons by interacting with a small hydrophobic pocket on the receptor protein, rather than with membrane lipids (*Proc. Natl. Acad. Sci. USA* **91:** 8200, 1994). Using the perforated-patch recording technique (nystatin 150 mg ml^{-1} in the recording pipette), we found that the amplitude of the current activated by 2.5 µM ATP was decreased by the extracellular application of 100 mM ethanol by 40 ± 5% (n = 8 cells). This degree of inhibition was similar to that observed in experiments using whole-cell patch-clamp recording. The inhibition was not significantly altered by the extracellular preapplication of 100 nM phorbol 12-myristate 13-acetate (PMA) for 8 - 10 min (n = 4 cells), 1 µM staurosporin for 4 - 8 min (n = 4 cells), or 5 - 50 µM forskolin for 4 - 8 min (n = 5 cells), respectively ($P > 0.05$). These results suggest that protein kinase A or protein kinase C is not involved in the inhibition of ATP-activated current by ethanol in these neurons.

713.2

Reduced Ethanol Sensitivity of Recombinant NMDA Receptors Under Divalent Cation-Free Conditions. Toorai Mirshahi* and John J. Woodward. Department of Pharmacology and Toxicology, Box 980524 Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Although ethanol has been shown to inhibit recombinant NMDA receptors, no specific site of action has been defined. We have previously shown that mutant NMDA receptors with absent or decreased Ca^{++} permeability [NR1(N616R, N616Q)] display a reduced ethanol sensitivity. To further investigate the possible interaction of ethanol and the Ca^{++} permeation site of the NMDA receptor, wild type and Ca^{++} impermeable mutant NR1 subunits (N616R) were tested for their ethanol sensitivity under different ionic conditions. The wild type and mutant NR1 subunits were expressed along with the NR2A or the NR2C in *Xenopus* oocytes. NMDA-induced currents were measured using conventional two-electrode voltage-clamp methods. Recordings were performed in Mg^{++}-free normal frog ringers with 1.8 mM Ba^{++} (Ba-NFR) or divalent cation-free ringers supplemented with 2 mM polyvinylpyrrolidone (PVP-NFR). Cells were clamped at -80 mV and currents were recorded in the presence of 100 µM NMDA and 10 µM glycine with varying concentrations of ethanol. Mutant NR1/2A receptors in Ba-NFR as well as wild type NR1-2A in PVP-NFR showed significantly reduced inhibition by ethanol compared to the wild type NR1/2A in Ba-NFR. Wild type and mutant NR1/2C in Ba-NFR or PVP-NFR displayed similar sensitivity to inhibition by ethanol when compared to the mutant NR1/2A receptors. Together, these results suggest that Ca^{++} permeability of NMDA receptors may influence the sensitivity of this receptor to inhibition by ethanol. Supported by NIAAA 08089 and NIDA 07027.

713.3

FUNCTIONAL PROPERTIES AND ETHANOL SENSITIVITY OF ε_4/ζ_1 NMDA RECEPTOR SUBUNITS EXPRESSED IN *XENOPUS* OOCYTES.

C. Wu*, F. F. Weight and K. Masood. Lab. Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205.

The ε4 subunit of the NMDA receptor is expressed in the embryonic brain, but its expression diminishes two weeks after birth, suggesting that it may play a role in early neural development. To study the properties of the subunit combination ε4/ζ1, we used two-electrode voltage-clamp recording in the *Xenopus* oocyte expression system. The ε4/ζ1 subunits were found to have a high affinity for NMDA, with an EC_{50} of 3.3 μM. The affinity for NMDA was about ten times lower than that for glutamate. The NMDA-activated current in ε4/ζ1 subunits was blocked by Mg^{2+} in a voltage- and concentration-dependent manner with an IC_{50} of 156 μM. These results indicate that the ε4/ζ1 subunits, like the ε3/ζ1 subunits, have a high resistance to Mg^{2+} block. The ε4/ζ1 subunits were sensitive to Zn^{2+} inhibition with an IC_{50} of 47 μM. No potentiation by low concentrations of Zn^{2+} was observed. Ethanol at 100 mM decreased the E_{max} of the concentration-response curve for NMDA-activated current without significantly affecting the EC_{50}. An increase in the inhibition of NMDA-activated current was observed when the concentration of ethanol was increased from 10 to 250 mM. The percentage inhibition of NMDA-activated current by 10, 25, 50, 100 and 250 mM ethanol was 2.8, 9.5, 16.5, 28.4 and 56.8%, respectively. However, compared with the ε1/ζ1 subunits, the ε4/ζ1 subunits were found to be less sensitive to ethanol inhibition. The observations indicate that the ε4/ζ1 subunit combination is similar to the ε3/ζ1 subunit combination in ethanol sensitivity and several functional properties.

713.4

ETHANOL AUGMENTS GABA-INDUCED CHLORIDE CURRENTS IN CULTURED HIPPOCAMPAL NEURONS. N. T. Rodgers-Neame*, K. E. Isenberg, and C.F.Zorumski, Dept. of Psych., Washington Univ. Sch. of Med., St. Louis, MO 63110 and Dept. of Neurol., Univ. of S. Florida Col. of Med., Tampa, FL 33612

Apparently similar neurons are affected differentially by ethanol. The role of GABAergic mechanisms in the action of ethanol in the brain is particularly interesting because of the wide variations in effects. Our study shows that ethanol has a rapid effect on some hippocampal neurons, while other hippocampal neurons remain resistant.

Electrophysiologic potentiation of the $GABA_A$ choride channel by ethanol was studied using whole cell patch clamp methods in primary neuronal cultures of neonatal hippocampus. Activation of the chloride current by ethanol requires the presence of GABA, and can be detected at a GABA concentration of 0.1 to 0.5 μM. The concentration of ethanol required for measureable augmentation of the GABA induced current was 5 mM and maximum augmentation was observed to occur at approximately 60 mM. In addition, only 21% of neurons responding to GABA also responded to ethanol and GABA, independent of concention of either compound, the diameter of the neuron or the age of the animal.

The implications of these results are that 1) the effect on the chloride current by ethanol in hippocampal neurons is GABA dependent 2) the effects are dependent upon specific qualities of the individual neuron - most likely subunit dependent and 3) these concentrations are well within the range noted to induce a physiologic effect in whole animals, indicating that chloride induced hyperpolarization of hippocampal neurons may be an important factor in the physiologic effects of ethanol.

713.5

DIFFERENTIAL SENSITIVITY TO ETHANOL OF GLYCINE-ACTIVATED CURRENTS IN HYPOTHALAMIC AND CORTICAL NEURONS. D. Schiller*, J.-H. Ye, & J.J. McArdle. Departments of Pharmacol. & Anesthesiol., New Jersey Med. Sch. (UMDNJ) & Grad. Sch. Biomed. Sci., Newark, NJ 07103-2714.

We examined the effect of ethanol on glycine-activated currents (I-Gly) of hypothalamic (VMH) and cortical neurons freshly isolated from young mice. I-Gly of 18 VMH and 29 cortical neurons was recorded with the nystatin perforated patch technique. Holding potential was -60mV and a fast superfusion system delivered varying concentrations of glycine (Gly) alone or in combination with 21.6, 43.2, or 64.8mM ethanol. Peak I-Gly was -227.04±71.06 pA (mean±SEM) for 5 VMH neurons exposed to 100μM Gly. When 64.8 mM ethanol was co-applied to these neurons, I-Gly increased to -471.06±134.84 pA. In contrast, for two other VMH neurons exposed to 100μM Gly and 64.8mM ethanol I-Gly decreased. Likewise, two populations of VMH neurons were detected following exposure to 43.2 mM ethanol; peak I-Gly increased and decreased for 2 neurons out of 4 examined. Similar results were obtained from VMH neurons exposed to 200μM Gly and 21.6, 43.2, 64.8 mM ethanol. For 8 cortical neurons 100μM Gly produced a peak I-Gly of -243.65±61.7 pA. When 64.8 mM ethanol was co-applied to these neurons, I-Gly increased ($p<0.05$) to -352.4±80.99 pA. Likewise, 21.6, 43.2, or 64.8mM ethanol consistently enhanced I-Gly of all other cortical neurons exposed to 100, 200 or 300μM Gly. These differential responses of I-Gly to relevant concentrations of ethanol suggest that while two populations of Gly receptor exist in the VMH, the cortex is homogeneous in this regard. Supported by grants NIAAA AA08025 and NIH NS31040)

713.6

ETHANOL INHIBITS RECOMBINANT α7 NICOTINIC ACETYLCHOLINE RECEPTOR-MEDIATED CURRENT IN *XENOPUS* OOCYTES. Dahong Yu, Li Zhang and Forrest F. Weight*, Lab. of Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205.

The α7 nicotinic acetylcholine receptor (nAChR) subunit has been cloned and characterized; it has the ability to form functional homomeric channels when expressed in *Xenopus* oocytes (*Neuron* 5:35-48, 1990). In addition, it is found in the mammalian CNS. Because of its high permeability to Ca^{2+}, it has been suggested that this subunit may be involved in the phenomena of neuronal plasticity, neural development and learning. Previous studies have shown that ethanol can potentiate nAChR mediated responses at the neuromuscular junction (*J. Physiol.* 244:409-429, 1975). Little is known, however, about the effects of ethanol on neural nAChRs. We expressed homomeric α7 nAChR cRNA in *Xenopus* oocytes and studied the effect of ethanol on the function of these receptors using the two-electrode voltage-clamp technique. We found that ethanol concentrations from 10 mM to 100 mM could produce a concentration-dependent decrease in the amplitude of α7 nAChR-mediated current. Maximal inhibition was observed at an ethanol concentration of 100 mM; maximal inhibition was 63% of control current induced by 10 μM (-)-nicotinic acid. The IC_{50} was 50 mM and the apparent Hill coefficient was 1.6. The observations indicate that ethanol modulation of α7 nAChR-mediated current differs from the effect of ethanol reported previously for nAChR at the neuromuscular junction .

713.7

ALIPHATIC ALCOHOLS EXHIBIT A CUTOFF IN POTENCY FOR ENHANCEMENT OF GABA-ACTIVATED ION CURRENT. Robert W. Peoples* and Forrest F. Weight. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892.

Intoxicating potency of straight-chain aliphatic alcohols increases as the number of carbons are increased up to 6 - 8 (the "cutoff"), then decreases despite increasing lipid solubility and membrane disordering potency (J. Pharmacol. Exp. Ther. 218: 669, 1981; Neuropharmacol. 17: 451, 1978). We have previously demonstrated that *n*-alcohols also exhibit a cutoff for inhibition of ion current activated by *N*-methyl-D-aspartate (NMDA) at 6 - 8 carbon atoms (Proc. Natl. Acad. Sci. USA 92: 2825, 1995). In this study, we investigated whether a cutoff for potentiation of $GABA_A$ receptors by *n*-alcohols could be observed in mouse hippocampal neurons in primary culture using the whole-cell patch-clamp technique. We found that the potency of alcohols for enhancing GABA-activated current increased as the carbon chain length was increased up to 11 - 12 carbons, but that maximally-attainable concentrations of alcohols with more than 12 carbon atoms did not enhance GABA-activated current. This cutoff thus differs from those previously reported for behavioral measures of alcohol intoxication.

713.8

EFFECT OF PENTANOL ON RECOMBINANT ACETYLCHOLINE RECEPTOR CHANNELS EXPRESSED IN HEK-293 CELLS. A. Ravindran*, K. Masood and F. F. Weight. Laboratory of Molecular & Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205.

The nicotinic acetylcholine receptor (AChR) of adult skeletal muscle is a channel-forming glycoprotein consisting of four homologous subunits assembled into a $\alpha_2\beta\delta\epsilon$ pentamer. The modulatory effects of 1-pentanol on acetylcholine (ACh)-induced current was studied in HEK-293 cells transiently transfected with mouse $\alpha\beta\delta\epsilon$ AChR subunit cDNAs. 30-40 hr after transfection whole-cell and outside-out patch-clamp techniques were used to record ACh-induced inward current from cells expressing AChR. ACh alone and in combination with different concentrations of 1-pentanol were applied to the recorded cell or outside-out patch using a rapid perfusion system that enabled complete exchange of solutions in 0.8-4 ms. ACh activated rapidly desensitizing inward currents with an EC_{50} of 13 μM. Co-application of 1-pentanol (0.5-16 mM) increased the amplitude of current activated by 2 μM ACh in a concentration-dependent manner. The potentiation was between 28-150% of the control response. The rate of AChR desensitization was also significantly increased (3-5 fold) by pentanol in a concentration-dependent manner. At agonist concentrations ≥ 5 μM, low concentrations of pentanol caused potentiation and high concentrations caused inhibition of the peak current. Further studies are underway to elucidate the mechanism of pentanol action on AChR channels.

713.9

INTERACTION OF GLYCINE AND ETHANOL ON RECOMBINANT HETEROMERIC NMDA RECEPTOR SUBUNITS.

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Glycine, a co-agonist of NMDA receptor channels, has been shown to potentiate NMDA-activated ion currents in heteromeric combinations. There is controversy regarding whether glycine can reverse ethanol's inhibitory effects on NMDA receptor channels. To address this issue and to understand the mechanism of action of ethanol at a molecular level, we examined the interaction of glycine and ethanol on ε1ζ1, ε2ζ1, ε3ζ1 and ε4ζ1 subunit combinations expressed in *Xenopus* oocytes. Membrane current activated by NMDA was recorded using the two-electrode voltage-clamp technique at a holding potential of -70 mV. Ethanol (100 mM) decreased E_{max} of the glycine concentration-response curve for all four heteromeric combinations. The inhibition of E_{max} by 100 mM ethanol for ε1ζ1, ε2ζ1, ε3ζ1 and ε4ζ1 combinations was 43.8, 15.0, 19.1 and 26.9%, respectively. However, ethanol did not significantly affect either the EC_{50} or the apparent Hill coefficient of these curves (ANOVA, $p>0.05$). The results indicate that ethanol is not competitive with glycine for the ε1ζ1, ε2ζ1, ε3ζ1 and ε4ζ1 subunit combinations.

713.10

ALIPHATIC *N*-ALCOHOLS EXHIBIT A CUTOFF IN POTENCY FOR THE INHIBITION OF RECOMBINANT GLUR3 RECEPTOR SUBUNIT CURRENT IN *XENOPUS* OOCYTES.

B. Emmanuel Akinshola* and Forrest F. Weight. Lab.Molecular and Cellular Neurobiology, NIAAA, NIH, Bethesda, MD 20892-8205.

The potency of straight-chain aliphatic alcohols for affecting the function of ATP-, NMDA- and 5-HT3-receptor gated ion channels increases as the number of carbons are increased up to a point called the "cutoff", where the potency decreases despite increased hydrophobicity of the alcohol (*PNAS* 91: 8200, 1994; *PNAS* 92: 2825, 1995; *Soc. Neurosci .Abst.*. 20: 1127, 1994). Although non-NMDA excitatory amino acid receptor-mediated responses have been reported to be inhibited with increasing potency with short-chain aliphatic *n*-alcohols from methanol to pentanol (*JPET* 262: 487, 1992), it has not been determined whether non-NMDA receptors exhibit a cutoff phenomenon. To address this question, we used the two-electrode voltage-clamp technique to study the effect of a series of straight-chain aliphatic alcohols on recombinant AMPA-type GluR3 receptor subunits expressed in *Xenopus* oocytes. We found that for short-chain alcohols from methanol to hexanol, potency for inhibition of kainate-activated current increased in proportion to the chain-length or hydrophobicity of the alcohol . However, potency decreased with heptanol and octanol, and nonanol did not inhibit kainate-activated current. The observations indicate that straight-chain aliphatic alcohols exhibit a cutoff in their potency for inhibition of non-NMDA glutamate receptors.

SODIUM CHANNELS I

714.1

SODIUM CHANNEL DENSITY IS INCREASED IN RAT DORSAL ROOT GANGLIA FOLLOWING CHRONIC CONSTRICTION INJURY (CCI)

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Neuropathic pain caused by peripheral nerve injury is associated with a persistent spontaneous discharge of injured fibers. This abnormal firing may be due to an accumulation of Na channels in peripheral sensory neurons. To test this hypothesis, we have used quantitative [^{3}H]saxitoxin ([^{3}H]STX) autoradiography to examine the distribution, density (B_{max}) and affinity (K_D) of Na channels in rats following CCI. Brains, spinal cords and dorsal root ganglia (DRG) were harvested from 6 normal rats and 6 rats 30 days after unilateral CCI. [^{3}H]STX autoradiography was performed on 20 µm sections through cortex, midbrain periaquaductal gray, lumbar spinal cord, and L_5-L_6 DRGs. Saturation isotherms were best fit to a model describing a single class of binding sites with K_Ds ranging from 0.7 - 3.9 nM and B_{max} from 157 - 720 fmol mg^{-1} tissue equivalent (t.e.). Na channel density was increased in the DRG ipsilateral to the nerve injury (B_{max}=248.1 ± 14.4 fmol mg^{-1} t.e.) compared with the sham side (205.7 ± 7.9) or normal DRG (190.1 ± 20.5) ($p < 0.05$). No differences were found in other regions. In summary, CCI caused an increase in [^{3}H]STX binding sites in DRG neurons that persisted for 30 days after the injury. This reflects an increase in the density of STX-sensitive Na channels, and suggests a mechanism for the effectiveness of sodium channel blockers in neuropathic pain.

714.2

REGULATION OF SODIUM CHANNEL mRNAs IN A NEUROPATHIC PAIN MODEL.

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Injury to the sciatic nerve evokes spontaneous electrical activity at high frequency. We have used a rat model where the sciatic nerve is loosely ligated to investigate the regulation of sodium channel mRNAs. A neuropathic pain condition ensues the loose ligature of the sciatic nerve. This syndrome includes symptoms of thermal and mechanical hyperalgesia and cold allodynia. The lumbar 4 and 5 dorsal root ganglia (DRG) from the ligated (right) and sham sides (left) were collected at different times following the loose ligature surgery. In addition to the central sodium channels mRNAs, the peripheral tissue expresses transcripts encoding novel channels. The mRNA levels of sodium channels in the above tissues were analyzed by QRT-PCR (Quantitative Reverse Transcriptase-Polymerase Chain Reaction). A unique external standard cRNA comprising a part of the 3'-UTR was synthesized for each of the brain type I and III and peripheral sodium channels. The complex regulation of sodium channel mRNAs following injury and its possible physiological significance will be discussed.

714.3

PROLONGED O_2 DEPRIVATION ALTERS Na^+ CHANNEL mRNA LEVEL IN EXCITABLE TISSUES IN THE DEVELOPING RAT.

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We have previously observed an increase in saxitoxin (a specific Na^+ channel ligand) binding sites in immature rat brains after being subjected to chronic hypoxia, suggesting that prolonged O_2 lack can regulate Na^+ channels in excitable tissues. In the present study, we asked whether Na^+ channel mRNA is regulated by O_2 availability in excitable tissues of the developing rat. Newborn rats (postnatal 0-3 day) were exposed to low O_2 environment (9.5±0.5%) for 27-30 days and then the brain, skeletal muscle and diaphragm were quickly removed for RNA extraction (n=4-9). Control litters were maintained in room air and studied simultaneously with the experimental rats. For the Northern blots, 20 µg of RNA was loaded in each lane on 1% agarose/formaldehyde gel. For the slot blots, serial concentrations (0.5-10 µg) of RNA were separately loaded in each slot. The cDNA probe is a 307 bp piece of Na^+ channel II α-subunit cDNA (from 4836 to 5142 bp) and is common all Na^+ channels (I, II, IIa, III). Northern blots show a clear band with a size of ~9.4 kb. Quantitation of slot blots shows that Na^+ channel mRNA markedly increased in brain (+80%) and skeletal muscle (+100%) in experimental rats; in contrast, no increase in mRNA was found in diaphragm with hypoxia. We conclude that long term hypoxia modulates Na^+ channel mRNA differently in various excitable tissues of developing rats (Supported by NIH grant P01 HD32573, HL 39924, NS 32578, and a UCP grant).

714.4

AUTORADIOGRAPHIC LOCALIZATION OF RADIOIODINATED δ-CONOTOXIN TXVIA BINDING SITES IN RAT BRAIN.

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Sodium channels are ubiquitously expressed in excitable cells within the nervous system. They are believed to exist as multiple subtypes which may exhibit select properties and unique neuroanatomic distributions. In order to better understand functional differences between these classes, defining their distribution and localization in the central nervous system is essential. We have employed a radioiodinated conotoxin with sodium channel specificity to pursue this question.

δ–conotoxin TxVIA, originally isolated from the molluscivorous cone snail Conus textile, slows sodium channel inactivation presumably by interacting with a distinct site on sodium channels. A radioiodinated analogue of this δ-conotoxin was applied in an autoradiographic assay. Sagittal sections of rat brain were labeled with [125I] δ–TxVIA and specific binding determined by competition with unlabelled δ-TxVIA. Autoradiograms were generated and intensities of binding were quantitated using computerized image analysis.

A reproducible pattern of [125I] δ-TxVIA binding sites was identified, with the highest density of labelling present in the choroid plexus. In declining order of abundance, sites were also seen in Purkinje and molecular cell layers of the cerebellum, pyramidal layer of hippocampus, granule cell layer of dentate gyrus, and with substantially lower levels in the outer layers of the cerebral cortex and striatum. Specific binding was displaceable by other delta (GmVIA, PVIA) and µO (MrVIA) conotoxins which are presumed to share a common site on the sodium channel. This displacement was not seen with other sodium channel ligands such as µ-conotoxin GIIIA.

These results indicate that the δ-conotoxin TxVIA identifies a unique neuroanatomic pattern of binding sites within the rat brain which suggest the existence of a unique set of functionally distinct sodium channel subtypes.

714.5

REGULATION OF Na CHANNEL mRNA IN NEUROBLASTOMA CELLS. Fred N. Quandt*. Dept. Molec. Biophys. and Physiol. Rush Univ., Chicago, IL 60612.

Little is known about the stimuli or mechanisms for regulation of Na channel mRNA in neurons. Physiological signaling pathways which may alter transcription of Na channel mRNA are being identified using cultured N1E-115 neuroblastoma cells as a model system. Messenger RNA is isolated and measured using an assay in which reverse transcription is followed by a competitive polymerase chain reaction. Previously we have reported that Na channel membrane current and mRNA are down-regulated in differentiated neuroblastoma cells grown in a Ca ionophore (A23187 or ionomycin, Quandt, Hirsh, and Sievert, Neurosci. Abs. 20:1506, 1994). This effect was selective since Kv 3.1 was not reduced under this condition. The result suggests that internal Ca alters transcription or lifetime of Na channel mRNA. The effects of other agents expected to alter internal Ca are being studied. Growth of cells in either nitrendipine, BAY K 8644, or depolarization with high K in the presence of valinomycin did not result in changes in Na channel mRNA abundance. Open Ca channels therefore appear to contribute little to regulation of mRNA under the normal growth condition. Addition of 40 μM veratridine to the culture medium for 3 days reduced Na channel mRNA by 80%. The action of veratridine on mRNA may be mediated by an increase internal Ca following activation of Ca channels after depolarization, or more likely following a decrease in Ca efflux through Na/Ca exchange. Supported by the National Multiple Sclerosis Society.

714.6

TRANSCRIPTIONAL REGULATION OF HUMAN BRAIN SODIUM CHANNEL GENES: PRESENCE OF β1 MESSAGE IN LA-N-5 HUMAN NEUROBLASTOMA CELLS. M.L. Beckman* and G.B. Brown. Psychiatry and Behavioral Neurobiology and The Department of Molecular Genetics and Biochemistry, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Sodium channels from central neurons consist of a heterotrimeric complex of α, β1 and β2 subunits. Both the rat and human genes for the β1 subunit have been cloned. The β1 subunit modifies the sodium channel by accelerating inactivation, shifting the voltage-dependence of inactivation to more negative membrane potentials and increasing the peak amplitude of sodium current.

Our interest is in understanding the transcriptional regulation of the human brain sodium channel genes. The human neuroblastoma cell line LA-N-5 has been shown to express sodium channels based on sodium flux and saxitoxin binding assays in this laboratory (Lombardo and Brown, 1992). In addition, data from ligase detection reaction assays in this laboratory confirm the presence of α subunit subtypes II and III in these cells, but not subtype I. Weiss and Sidell have also described sodium conductances in these cells using the patch clamp technique (Weiss and Sidell, 1991).

We have extended these observations to show that these cells also express message for the β1 subunit. PCR amplification of DNase treated reverse transcribed mRNA from LA-N-5 cells was carried out and revealed the expected sized product. Currently, primer extension and 5'-RACE experiments are being conducted to map the transcriptional start site. The LA-N-5 cell line will be useful in our studies of the β1 subunit of human brain sodium channel and factors that may regulate its expression. (Supported by USPHS grant DA07237)

714.7

IMMUNOCYTOCHEMICAL AND ELECTROPHYSIOLOGICAL MEASURMENTS OF SODIUM CHANNEL DISTRIBUTION ON UNINNERVATED SKELETAL MYOTUBES. B.D. Anson*, and W.M. Roberts. Institute of Neuroscience, University of Oregon, Eugene, OR. 97403.

Vertebrate neuromuscular transmission depends, in part, on high densities of acetyolcholine receptors (AChRs) and voltage-gated sodium (Na^+) channels at the endplate. Much is known about the localization of AChRs to the endplate, but much less is known about Na^+ channels. To begin to understand how the specialized distribution of Na^+ channels on a muscle cell develops and is maintained, we have used immunoflurescent and electrophysiological methods to study the distribution of Na^+ channels on uninnervated chick skeletal myotubes growing in cell culture.

We labelled chicken myotubes with an affinity purified polyclonal antibody directed against the putative inactivation loop of the Na^+ channel, and then visualized the primary antibody distribution with a biotinylated secondary antibody and an avidin-conjugated fluorophore. Control myotubes were processed in parallel with experimental myotubes but without addition of primary antibody. The distribution of antibody labelling was examined by comparing blindly chosen areas of experimental and control myotubes.

Most areas on experimental myotubes showed no significant label over control. However, 18% (20/112) experimental areas showed label intensity greater than 2 standard deviations above the control mean. Furthermore, only 18% (15/82) of the patches containing nuclei showed significant labelling.

By using a tight-seal whole cell electrode to record currents focally elicited by a loose-seal patch electrode, we were able to record currents from 23 patches of membrane separated by 10-100μm from six different myotubes. For all but one patch, peak Na^+ current for each patch on a cell was between 0.5 and 1.5 of the mean for all patches from that cell.

These results suggest that: 1). Na^+ channels are distributed non-uniformly over uninnervated myotubes but that the distribution does not have a sharp boundary, and 2). Nuclei within a single cell may differentially express unequal amounts of Na^+ channel.

714.8

APPEARANCE OF SODIUM CURRENTS IN *XENOPUS* EMBRYONIC MYOCYTES MAY BE INDEPENDENT OF INNERVATION. E. Prabhakar and A.E. Spruce* Dept. of Pharmacology, Univ. of Birmingham, Birmingham, B15 2TT. UK.

Embryonic *Xenopus* myocytes are functionally innervated and able to generate sodium-dependent action potentials *in vivo* within 12 hr from Stage 15 (when morphological and functional differentiation of muscle begins). In neonatal rat muscle, sodium current (I_{Na}) expression is regulated by innervation. Therefore, by comparing the expression of I_{Na} in myocytes isolated either at St. 15 ("aneural") or at St. 24 soon after they had been innervated ("neural"), we were able to assess the role of nerve in this aspect of electrical differentiation. The timing of the whole-cell patch clamp recordings (relative to St. 15) from "aneural" and "neural" myocytes was equivalent 24.8 ± 0.5 hr (n = 17) and 20.8 ± 2.0 hr (n = 18), respectively. Although a higher proportion of "neural" myocytes expressed I_{Na} (61% (11/18) vs 41% (7/17)), its density was not significantly different: 62.1 ± 23.3 pA/pF ("neural", n = 18); 25.1 ± 8.2 pA/pF ("aneural", n = 17; $p < 0.2$). The expression of I_{Na} was found to be dependent on extracellular Ca^{2+}, however. When "aneural" myocytes were cultured in 0 mM Ca^{2+} (the recording solution which was spritzed onto the cells still contained Ca^{2+}), I_{Na} density was significantly reduced (5.9 ± 3.2 pA/pF; n = 11; $p < 0.03$), although the proportion of cells containing I_{Na} was not altered (45%; 5/11).

Therefore, the early expression of I_{Na} in developing myocytes appears to be a cell autonomous mechanism, possibly triggered by "muscle-derived factors" and involving Ca^{2+} entry. Nerve, however, may play a role in maintaining an accentuated level of I_{Na} (Corfas & Fischbach, 1993. J.Neurosci. 13:2118).

714.9

EXPRESSION OF mRNA FOR A SODIUM CHANNEL IN SUBFAMILY 2 IN SPINAL SENSORY NEURONS. S.G. Waxman and J.A. Black*. Dept. of Neurology, Yale Univ. Sch. of Med., New Haven, CT and Neuroscience Res., VA Med. Ctr, West Haven, CT 06516

Spinal sensory neurons (dorsal root ganglion [DRG] neurons) display a variety of voltage-sensitive sodium currents that differ in terms of kinetics and voltage-dependence. The expression of these different currents suggests the presence of multiple types of Na^+ channels in these cells. Previously we have demonstrated that adult DRG neurons express mRNA for rat brain Na^+ channel α-subunits I and II/IIA. In the present study we have used RNA blot and non-radioactive *in situ* hybridization methods with probes generated from the sequence of NaG, a putative glial Na^+ channel that appears to belong to Na^+ channel subfamily 2, to examine the expression of Na^+ channel mRNA from this subfamily in spinal sensory neurons and Schwann cells within DRG.

RNA blots showed a major band at ~7.5 kb in postnatal day 2 (P-2) and adult DRG RNA with the NaG probe, while no hybridizable RNA was detected in P-2 or adult brain or kidney samples.

In situ hybridization detected mRNA hybridizing with the NaG riboprobe within DRG neurons and Schwann cells *in vivo*, with higher levels of expression in the former. In contrast, hybridization with the NaG probe was not detected in neurons within the hippocampus, cerebellum or spinal cord. The expression of the mRNA hybridizing with the NaG probe appears to be developmentally-regulated in both Schwann cells and DRG neurons, with levels increasing as development proceeds. Thus, in addition to the mRNAs for rat brain types I and II/IIA α-subunits, the mRNA for an additional Na^+ channel belonging to subfamily 2 is expressed in DRG neurons.

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714.10

DIFFERENTIAL DISTRIBUTION OF HUMAN BRAIN SODIUM CHANNEL SUBTYPE III mRNA: ANALYSIS BY LIGASE DETECTION REACTION (LDR) C.N. Lin* and G.B. Brown. Psychiatry and Behavioral Neurobiology, Univ. of Alabama at Birmingham, Birmingham, 35294

In a previous study a reverse transcriptase-polymerase chain reaction (RT-PCR) coupled ligase detection reaction (LDR) assay was used to detect the differential distribution of human brain sodium channel (HBSC) subtype I and II mRNA by quantifying the relative amounts of subtype I to II radio-labeled LDR product (Lu et al., 1992). However, information concerning HBSC III has been lacking. The RT-PCR-LDR analysis has now been extended to examine the distribution of subtype III mRNA in human brain. First total RNAs were extracted from seven regions (frontal, temporal and occipital cortex, cerebellum, spinal cord, basal ganglia and hippocampus) of different individual normal human brains and targeted mRNA fragements were amplified by RT-PCR using a pair of primers that are common to both III and I subtypes. Then a common ^{32}P-labeled primer (20mer) along with two subtype specific primers (30mer for III and 25mer for I, both abutting the 5' end of the common primer) were used in LDR reactions carried out for 30 cycles of 94°C for 1.5 min followed by 45°C for 4 min. The expression of subtype-specific mRNA can be quantified by densitometric analysis of the different-sized radiolabeled LDR products on autoradiograms. The results reiterate that LDR can provide a sensitive, quantative assay for measurement of total RNA containing allele-specific transcripts differing only by a single nucleotide. The data showed that HBSC III mRNA is differentially distributed in human brain compared to subtypes I and II, suggesting that they are differentially regulated. The relative ratio of III to I mRNA varies markedly from a low value of 0.49±0.07 in occipital lobe to a high of 2.92± 0.39 in cerebellum.

714.11

Expression of Na channel 6 alpha subunit mRNA in the developing rat central nervous system. K.L. Schaller and J.H. Caldwell*
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A novel sodium channel isoform (NaCh 6) has been isolated and characterized from rat brain (Schaller et al, J. Neurosci, 1995). Previous studies showed that transcripts for NaCh 6 are abundant in the adult rat brain and by *in situ* hybridization are expressed in both neurons annd glia. Here the regional distribution of NaCh 6 mRNA expression in the developing rat brain was investigated by *in situ* hybridization. At embryonic days 15 and 18 weak hybridization is detectable over developing neuroepithelium. Transcript levels increase during postnatal development throughout the brain and spinal cord. By P1 NaCh 6 mRNA is detected in the pyramidal cells and dentate gyrus of the hippocampus, the granule cells of the cerebellum, and throughout the olfactory bulb. In these regions the levels of expression become progressively greater with maturation. In other areas of the CNS, for example the cortex, NaCh 6 transcript expression is highest at P7-P14 and then falls with further maturation. Expression of NaCh 6 transcripts in glial cells is seen beginning at P21 in both the brain and spinal cord. Thus it appears that the expression of NaCh 6 is both temporally and regionally regulated in the developing CNS.

714.12

CNS MYELINATION IS NOT REQUIRED FOR ESTABLISHMENT OF THE ADULT EXPRESSION PATTERN OF SODIUM CHANNEL SUBUNITS. P.A. Felts*, J.A. Black and S.G. Waxman. Dept. of Neurol., Yale Univ., New Haven, CT 06510 & PVA/EPVA Neurosci. and Regen. Res. Ctr., VAMC, W. Haven, CT 06516.

Rat brain sodium channels consist of a β_1-subunit, a β_2-subunit and an α-subunit. Currently there are at least 4 different α-subunits (forms I, II (Noda et al., *Nature* 320:188), III (Kayano et al., *FEBS Let* 223:417) and a putative glial subunit (NaG; Gautron et al., *PNAS* 89:7272)). During late embryonic-early postnatal development of the rat nervous system, the expression pattern of sodium channel subunits is altered: expression of form I and the β_1-subunit increases, and form III decreases. The adult expression pattern is attained at approximately 3 weeks postnatal age. Thus the developmental progression of sodium channel subunit expression is coincident with the period of most active myelination in the CNS. It is not known, however, if this progression is dependent upon myelination. We have therefore compared sodium channel α- and β_1-subunit mRNA expression in *myelin deficient* (*md*) rats and unaffected male littermates (20-22 days postnatal age). *md* is a sex-linked mutant of Wistar rats characterized by a near total lack of myelin in the CNS. Subunit expression was examined in the hippocampus, cerebellum, spinal cord and dorsal root ganglia (DRG) using *in situ* hybridization with digoxigenin labeled riboprobes.

The expression pattern we observed in unaffected animals was generally in accord with previous studies on adult animals. In the 3 CNS regions, the intensity of subunit expression was: II>β_1>I>III with no detected expression of NaG. In DRG neurons, α-subunits I and II and the β_1-subunit were prominently expressed, with less expression of α-subunit III. DRG neurons did express NaG mRNA. In *md* rats, the pattern of expression of sodium channel α-subunits I, II, III and NaG, and the β_1-subunit, was indistinguishable from the pattern observed in unaffected littermates in all tissues studied. This similarity indicates that CNS myelination is not necessary for progression from the embryonic to the adult sodium channel subunit expression pattern.

714.13

LOCALIZATION OF SODIUM CHANNEL-ENCODING mRNAs IN THE RAT CENTRAL NERVOUS SYSTEM. G.D. Pratt* and D.R. Riddall. Molecular Pharmacology, The Wellcome Foundation Ltd., Beckenham, Kent, BR3 3BS, United Kingdom.

Molecular cloning has revealed at least three subtypes of the α subunit of voltage-sensitive sodium channels in the rat brain, designated as I, II and III. We have employed *in situ* hybridization histochemistry to examine the distribution of mRNA species that encode sodium channel types I, II and III in the rat CNS. Complementary, subtype-specific 50-oligomeric deoxyribonucleotide probes were radiolabelled with α-[^{35}S]dATP using terminal deoxynucleotidyl transferase. Following hybridization, sections were washed under suitable stringency conditions and the specific microscopic transcript localization was resolved by dipping in Hypercoat™ LM-1 emulsion.

Sodium channel encoding mRNAs are differentially located throughout the brain. Only a weak general expression of type III mRNA was apparent in the adult rat brain although more concentrated levels were evident in the inferior olivary nucleus, nucleus of the solitary tract, locus coeruleus, medial habenula and also granule cells of the dentate gyrus. In contrast, expression of type II mRNA predominated in the cerebral cortex, the granule cell layer of the cerebellum, pyramidal cells of the hippocampus and dentate gyrus granule cells. Comparatively lower levels were found in the caudate putamen and thalamus. Type I mRNA expression is generally more pronounced in rostral structures with particularly high transcript levels being observed in motor neurones of the spinal cord, the spinal trigeminal nucleus, pontine nucleus, deep cerebellar nuclei, thalamic nuclei and layer III-IV of the somatosensory cortex. The anatomical substrates associated with type I mRNA may implicate the specific involvement of type I sodium channels with sensory spinal neurotransmission and also corticothalamic processing.

SODIUM CHANNELS II

715.1

MODELLING IMPLICATES A SPECIFIC SODIUM CHANNEL TRANSITION DEFECT IN EQUINE PERIODIC PARALYSIS. WJB Hanna[1,2]*, RG Tsushima[2,3] & PH Backx[2,3]. [1]Biophysics Group, Dept Physics, Univ Guelph, Guelph, ONT, N1G 2W1, Canada; [2]Cardiology, Dept Med., Univ Toronto School of Med. & [3]CCRW, The Toronto Hospital, 101 College St, Toronto, ONT, M5G 2C4, Canada.

Equine hyperkalemic periodic paralysis (HPP) is associated with a skeletal muscle sodium channel α-subunit mutation corresponding to Phe1412Leu in the rat μ1 sodium channel (Rudolph et al., Nature Genetics 2:144, 1992).

Current from μ1 channels containing this mutation differ from the wild-type in several ways: (1) whole-cell peak currents decay several-fold more slowly and decay incompletely, producing a pedestal of sodium current, (2) whole-cell peak currents recover from inactivation approx. one order of magnitude more rapidly, (3) the steady-state inactivation curve is shifted to the right with no change in slope, (4) single channels produce brief re-openings during maintained depolarizations, (5) mean single-channel open-time is increased by approx. 40-60%. Activation parameters are not affected.

The phenotypic differences between control and HPP sodium channels are predicted by moderate changes in rate constants (in the sodium channel gating model developed by Kuo and Bean [Neuron 12:819, 1994]) involving transitions between the open ("O") and open-inactivated ("I") states. Reductions of O_{ON} combined with increases of O_{OFF}, each of one order of magnitude or less, in the Kuo/Bean model reproduce precisely the whole-cell inactivation gating abnormalities observed with the equine HPP mutation. Monte-Carlo simulations also predict the single-channel experimental results. Alterations of rate constants representing other molecular transitions in this model do not predict the complete HPP phenotype. These results suggest that the cytoplasmic end of S3-DIV is critical for normal binding of the inactivation gate with its receptor.

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715.2

CYSTEINE MAPPING IN THE SELECTIVITY REGION OF THE SODIUM CHANNEL. S.-F. Chen*[1], H.A. Hartmann[1] and G.E. Kirsch[1,2]. Depts. of Molecular Physiology and Biophysics[1] and Anesthesiology[2], Baylor College of Medicine, Houston, TX 77030.

The pharmacology of brain and skeletal muscle sodium channels (NaChs) is different from that of the cardiac isoform, but the ionic selectivity is the same in these different types of NaChs. A conserved lysine residue in the SS2 segment of domain III is critical for selectivity in NaChs (Heinemann *et. al.*, 1992; *Nature 356:441-443*). A single point mutation from cysteine to tyrosine in the SS2 of the domain I of cardiac NaCh switches the pharmacology to the type of brain/skeletal muscle NaChs (Satin *et. al.*, 1992; *Science 256:1202-1205*). A combined approach by systematic cysteine substitution and application of cysteine-modifying compounds was carried out in the SS2 of domains III and IV of the switched NaCh to map the residues which may line the narrowest part of the pore.

The negatively charged (2-sulfonatoethyl)methanethiosulfonate (MTSES) increased the conductance of D1713C and decreased the conductance of W1712C, M1421C, and W1420C. These results indicate that the SS2 of domain III and IV are not symmetrically aligned. D1713 is at external vestibule while the other three are already inside the narrow region. MTSES cannot modify K1418C and A1710C. This suggests that these two residues are deep inside the pore where anions cannot reach. The positively charged (2-aminoethyl)methanethiosulfonate (MTSEA) can still modify K1418C and A1710C with very slow kinetics. This indicates that the large molecule cannot freely rotates in the vicinity of these two residues. Selectivity amongst alkali metal ions was drastically reduced in the K1418C mutant such that P_K / P_{Na} became 1.2, versus 0.1 in control. The sequence of selectivity for all the other mutants remained as the Eisenman sequence X or XI. These results suggest that the side group of K1418 contributes to much of the spatial restriction at NaCh selectivity filter.

715.3

TTX DIFFERENTIALLY MODULATES FAST INACTIVATION GATES IN ADRENAL CHROMAFFIN CELL NA^+ CHANNELS.
F. Horrigan & R. J. Bookman* Dept. of Mol. & Cell. Pharmacology, University of Miami, Miami, FL, 33101

Na^+ current inactivation in chromaffin cells can be described by a kinetic model that includes two parallel fast inactivation states (I_{rfast} & I_{rslow}) that differ primarily in their time course of recovery from inactivation ($\tau_{fast} \approx 8$ ms, $\tau_{slow} \approx 140$ ms @ -80 mV). I_{rslow} and I_{rfast} interact in a strictly competitive manner such that transitions between inactivated states do not occur unless channels first recover from inactivation. Repetitive depolarizations (5 ms @+20 mV) at 5 ms intervals increase the fraction of channels in I_{rslow} since many channels recover from I_{rfast} during a 5 ms interval while few recover from I_{rslow}. Measurements of cell membrane capacitance (C_m) can be used to monitor the number of channels in I_{rslow} (Horrigan & Bookman 1994 Neuron, 13). A 5ms depolarization (+20mV) produces a transient increase in C_m (ΔC_t) as Na channels inactivate and then recover from inactivation. The decay of ΔC_t matches the time course of recovery from I_{rslow}. Repetitive depolarization produces an increase in ΔC_t amplitude consistent with the observed increase in I_{rslow}. TTX (1μM) abolishes I_{Na} and slows the decay of ΔC_t by a factor of 8 but does not alter the ΔC_t amplitude after a 5 ms pulse. Thus TTX binding appears to retard recovery from I_{rslow} significantly without altering the number of channels that enter I_{rslow} during a 5ms depolarization. In contrast, recovery from I_{rfast} is not modulated by TTX since the repetitive pulsing paradigm still produces an increase in ΔC_t amplitude. Thus TTX and related toxins may provide important tools for distinguishing the molecular mechanisms underlying these two forms of fast inactivation.

715.4

ON THE ACTIVATION-INACTIVATION COUPLING IN SODIUM CHANNELS. C. Y. Lee*, Department of Physiology, National Yang-Ming University, Taipei, Taiwan, R.O.C. 11221

The fast sodium inactivation is known to couple with the charge movement leading to channel opening. However, the IFM cluster responsible for inactivation [1] contains no charged residues to interact with the gating charges. How could the gating charge movement cause the IFM cluster to plug the pore? We propose that D1487, located next to IFM, could be mediating the activation-inactivation coupling. In the resting state D1487 may make hodrogen bonding with the residues in a particular domain (called the inactivation-coupled domain). This hydrogen bonding prevents the IFM cluster from moving to plug the pore through hydrophobic forces. During channel activation, a tyrosine or tryptophan may become negatively charged, breaking the hydrogen bonding. The IFM cluster could then move to plug the pore. This model can also explain why batrachotoxin activates the sodium channel without causing the fast inactivation by assuming one of its oxygen triad binds near Y1497-Y1498 [2].

[1] Patton, DE, West JW, Catterall WA, et al. 1992. *PNAS.* **89:**10905-10914.
[2] Lee, C.Y. 1995. *Physical mechanisms of synaptic transmission.* Ho-Chi.

715.5

COMPARISON OF SLOW INACTIVATION IN WILD TYPE AND HYPP-T698M RAT SKELETAL MUSCLE Na^+ CHANNELS. T.R. Cummins*, W.S. Agnew+ and F.J. Sigworth. Interdept. Neurosci. Prog. and Dept. of Physiol., Yale Univ. Sch. of Med., New Haven, CT 06510 and +Depts. of Physiol. and Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

It has been hypothesized that slow inactivation must be compromised by mutations causing hyperkalemic periodic paralysis (HYPP) to account for the duration of the paralysis observed in patients with HYPP (R. Ruff, Biophys. J., 66:542-545, 1994). We are studying a mutation (T698M) in the rat skeletal muscle sodium channel that corresponds to one of the human HYPP mutations (T704M) and have compared slow inactivation of wild type and T698M channels stably expressed in HEK293 cells.

Wild type channels exhibit slow inactivation in HEK293 cells. Slow inactivation develops with a similar time course to that observed for sodium currents in rat skeletal muscle. As has been observed with other preparations, slow inactivation in HEK293 cells remains intact when fast inactivation is removed by chemical modification and coincides with a negative shift in the voltage dependence of gating currents. However, while in native rat muscle tissue slow inactivation occurs at more negative voltages than fast inactivation, in HEK293 cells the opposite is true.

T698M channels also exhibit slow inactivation in HEK293 cells. At negative potentials slow inactivation and recovery form slow inactivation occur with a similar time course to that of wild type channels. At depolarized potentials, slow inactivation develops more slowly and is much less complete for HYPP-T698M than for wild type channels. This indicates that slow inactivation is indeed defective in HYPP mutant sodium channels and may contribute to the maintenance of paralysis in patients with HYPP.

715.6

CHARACTERIZATION OF SODIUM-DEPENDENT PLATEAU POTENTIALS IN LEECH RETZIUS CELLS ISOLATED IN CELL CULTURE. J.D. Angstadt* and J.J. Choo. Department of Biology, Siena College, Loudonville, NY 12211.

Retzius cells, identified by their characteristic size and location, were removed from isolated ganglia of *Hirudo medicinalis*. Cells were plated in separate 35 mm petri dishes pretreated with concanavalin A or poly-L-lysine and containing L-15 medium with gentamicin. Most experiments were performed on neurons maintained in culture for 7-11 days. The isolated neuron was superfused with a variety of saline solutions and microelectrodes usually contained tetraethylammonium (TEA) to suppress K^+ currents. Isolated Retzius cells had resting membrane potentials (RMP) of -50 to -60 mV and were quiescent or fired impulses at a low rate. Prolonged plateau potentials (PP) were evoked by brief (1 s) depolarizing current pulses. The plateau depolarization stabilized near 0 mV and persisted for several minutes in some cells. The membrane potential repolarized to the normal RMP spontaneously or in response to a brief hyperpolarizing current pulse. PPs were associated with a conductance increase, persisted in salines containing 200 μM Cd^{2+} or where Ca^{2+} was replaced with Ca^{2+} channel blockers such as Co^{2+}, Ni^{2+}, or Mn^{2+}. In contrast, PP were rapidly and reversibly eliminated when Na^+ was replaced with an equimolar concentration of N-methyl-D-glucamine. In some cells, repetitive bursting activity occurred spontaneously or in response to injected current. These membrane potential oscillations were similar in many respects to those evoked in intact ganglia by salines containing calcium channel blockers.

715.7

CHARGE SENSITIVE LIGHT INDUCED SODIUM CHANNEL IN *HERMISSENDA* PHOTORECEPTOR M. Sakakibara*, T. Yoshioka, H. Inoue Lab. Neurobiol. Engr., Tokai Univ., Sch. of Hightech., Numazu 410-03, JAPAN, Lab. Mollec. Neurobiol., Waseda Univ., Sch. of Human Sci., Tokorozawa 359, JAPAN

Our previous study explained the two contradictory evidences on the mechanisms of invertebrate phototransduction; the process of PIP_2 hydrolytic breakdown into IP_3 and diacylgrycerol(DG) is necessary for the generation of photoresponse whereas the product, IP_3, is not requisite. We hypothesized that the local charge movement caused by PIP_2 hydrolysis near the inner surface of rhabdomeric membrane controls the gating of non-voltage dependent, light sensitive sodium channel. The hydrolytic charge liberation was confirmed on the suppression of photoresponse not only by the injection of charge chelators such as neomycin, spermine but stoppage of PI turnover by IBMX, LiCl and R59022. We postulated that the light sensitive sodium channel is activated in the charge sensitive manner. The indirect increase of pH inside the type B photoreceptor by perfusing NH_4Cl containing ASW augmented the initial component of photoresponse by 20 % increase in amplitude. On the other hand pH decrease by injection of acetate buffer(pH 4.6) maximally suppressed the photoresponse by 70% then returned to the original one within 20 min. The size of photoresponse was found to be proportional to the dissociation of phosphorus which was obtained by ^{31}P-NMR experiment. Here we assume that phospholipase C, hydrolysing PIP2, may be activated rapidly by membrane depolarization as observed in rat brain synaptosome. Since the voltage-dependent sodium channel in the squid axon has been known to be inactivated to the internal pH change, acidic/basic, it is likely that the B photoreceptor is activated by light in the membrane charge dependent fashion.

715.8

A NOVEL OUABAIN-SENSITIVE SODIUM ACTIVATED CATIONIC CURRENT OF FROG TECTAL NEURONES IN VITRO. A. Zaykin*, A.Nistri. Biophys. Lab, Int Sch Adv Studies (S.I.S.S.A.), 34013, Trieste, Italy.

Voltage clamp techniques based on whole cell patch recording were used to study transient voltage-activated currents of frog tectal neurones in a slice preparation at room temperature. The external solution contained oxygenated and buffered saline solution (100 mM NaCl). The pipette solution usually contained Cs sulfate (sometimes replaced by K gluconate) plus ATP, EGTA and 5-10 mM Na . In forty neurones held at -50 mV depolarizing voltage steps evoked a fast inward sodium current with threshold at about -40 mV and peak after 1 ms. Under these conditions sodium currents were always followed by a large transient outward current carried out in approximately equal degree by Cs or K ions with peak at about 3-5 ms. The same type of fast inward sodium current was also activated by command potentials more negative than -90 mV but in this case it was not followed by any outward component corresponding to the Cs current observed at depolarized potentials. Externally applied TTX (1 μM), ouabain (50 μM) or Cs (10 mM) simultaneusly blocked inward and outward current components induced by depolarizing command steps and also blocked the isolated sodium current evoked by voltage steps more negative than -90 mV. The present data suggest that frog tectal neurones possess a novel type of sodium activated transient cationic current probably characterized by a rapid change in the selectivity of the same channel from sodium to potassium/cesium following the initial entry of sodium into the channel itself. This current possesses some simillarity to the sodium/potassium pump current of peripheral cells but it seems to present the unusual properties of rapid kinetics and voltage-dependent activation.

715.9

DIFFERENTIAL PROPERTIES OF VOLTAGE-DEPENDENT Na^+ CHANNELS FOLLOWING AXOTOMY IN CUTANEOUS AFFERENT DRG NEURONS OF ADULT RAT. M.A. Rizzo, S.G. Waxman*, J.D. Kocsis. Dept. of Neurology, Yale School of Medicine, New Haven, CT 06510 and Neuroscience Research Center, VAMC, West Haven, CT 06516.

Studies of the behavior of Na^+ currents particular to cutaneous afferent dorsal root ganglion (DRG) neurons following chronic axonal injury should increase our understanding of hyperexcitability of these neurons following chronic axonal injury. Cutaneous afferent DRG neurons in short-term cultures were identified by retrograde fluorescence labelling of the sciatic nerve with Fluoro-gold. Using the whole-cell and bleb-patch-clamp recording techniques, Na^+ currents were recorded from these neurons pre (controls)- and 18 days post-axotomy and tested for sensitivity to 1 μM TTX and 100-400 μM lidocaine. Na^+ currents in neurons post-axotomy, in contrast to controls, tended to be blocked by TTX, and by relatively higher concentrations of lidocaine. Kinetically slow, TTX-resistant Na^+ currents predominated in control neurons. Predominantly kinetically fast, TTX-sensitive Na^+ currents emerge post-axotomy. Steady-state activation and inactivation curves constructed from Na^+ current recordings of somatic membrane blebs (fragments of membrane 3-8 μm diameter corresponding to 0.3-2 pF) excised from cutaneous afferent DRG neurons pre- and post-axotomy revealed that conductances post-axotomy had a decreased slope factor, broader window currents, and splay of steady-state inactivation in comparison either to slow Na^+ conductances in control neurons, or to kinetically equivalent (fast) counterparts in non-axotomized, non-cutaneous afferent DRG neurons. The results are consistent with the hypothesis that, following axotomy, new subpopulations of Na^+ channels occur on cutaneous afferent DRG neurons. This may confer altered neuronal excitability, contribute to ectopic impulse generation, and underly pain and paresthesias following chronic axonal injury.

715.10

SODIUM CHANNELS IN B104 CELL LINE: ELECTROPHYSIOLOGICAL PROPERTIES AND mRNA PROFILE. X. Q. Gu*, S. D. Dib-Hajj, J. A. Black and S. G. Waxman. Department of Neurology, Yale University School of Medicine, New Haven, CT. and Neuroscience & Regeneration Research Center, VA Medical Center, West Haven, CT. 06516.

B104 is an established neuroblastoma cell line which was reported to exhibit electrical excitability, but previous studies have not elucidated the Na current characteristics of these cells. We used whole-cell patch clamp, reverse transcription-PCR (RT-PCR) and *in situ* hybridization to examine the properties of Na current and the mRNA profile of the Na channel α and β1 subunits.

Whole-cell patch clamp studies of B104 cells maintained in DMEM media supplemented with 20% fetal calf serum showed Na currents in about 30% of pyramid-like or spherical cells but not in bipolar cells. Na current ranged up to 4.0 nA in amplitude. Voltage steps more positive than -60mV activated transient inward currents, and currents were largest at potentials close to -30mV. Ten nM TTX reduced sodium current amplitude by 20-60%.

RT-PCR followed by restriction enzyme analysis of the amplified products confirmed our published results that B104 cells express αIII subunit mRNA; αI and αII mRNAs were not detected. We now show that β1 subunit mRNA is also expressed in the B104 cells. *In situ* hybridization using antisense riboprobes specific for the various Na channel α and the β1 subunits demonstrated moderate-to-high levels of αIII and β1 mRNA expression in some B104 cells. The expression of Na channel αIII and the β1 subunits appears to provide the molecular basis for the TTX-sensitive, voltage-dependent Na current that is present in B104 cells.

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715.11

MUSCARINIC MODULATION OF SODIUM CURRENT BY ACTIVATION OF PKC IN RAT HIPPOCAMPAL NEURONS. A.R. Cantrell*, T. Scheuer, and W.A. Catterall, Department of Pharmacology, University of Washington, Seattle, WA 98195.

Voltage -gated sodium channels play an important role in neuronal excitability in the hippocampus but are infrequently recognized as targets for neuromodulation. Phosphorylation of brain sodium channels by protein kinase C (PKC) decreases peak current and slows macroscopic inactivation. Hippocampal muscarinic receptors activate PKC. Therefore, we have tested whether sodium channels can be modulated by muscarinic receptor activation in acutely-isolated adult (3-5 wks postnatal) hippocampal neurons using whole-cell voltage clamp recording.

Application of the muscarinic agonist, carbachol (20μM), reduced peak sodium current an average of 29.8 ± 2.5% in 28 /33 cells tested and slowed macroscopic inactivation at all potentials. No change in the voltage dependence of activation or inactivation was observed. These effects were mediated via PKC as they were eliminated when a specific PKC inhibitor peptide (PKC19-31) was included in the pipette solution and mimicked by the extracellular application of the PKC activator, OAG.

These results demonstrate that muscarinic activation of endogenous receptors on hippocampal neurons strongly modulates sodium channel activity by activation of PKC. Such modulation is expected to have potent effects on overall neuronal activity.

715.12

FRACTAL KINETICS OF IONIC CURRENTS IN BIOLOGICAL MEMBRANES. K.-D. Kniffki* and C. BRAUN. Physiologisches Institut, Universität Würzburg, Röntgenring 9, D-97070 Würzburg.

Fractal geometry has provided a mathematical formalism for describing complex spatial and dynamical structures in nature (B.B. Mandelbrot, The Fractal Geometry of Nature, 1983). It seems natural to apply a fractal analysis to protein kinetics involved in the gating mechanism of ionic channels in excitable membranes. In the classical papers of Hodgkin and Huxley the rate constants k are treated as textbook descriptions of macroscopic kinetics, i.e. as time-independent entities. Recently, T.G. Dewey, Proc. Natl. Acad. Sci. 91: 12101-12104, 1994, described the exchange of protons in tritiated lysozyme in a restricted (fractal) geometry including concentration fluctuations by using $k(t) = k't^{-\lambda}$. Here k' is a time-independent constant and the scaling exponent λ is restricted to values between 0 and 1. By using this particular approach for the Hodgkin and Huxley gating "particles" $\mu = m, h, n, \ldots$ between their 'closed' and 'open' states, i.e. $k_\alpha(t) = k'_\alpha t^{-\lambda_\alpha}, k_\beta(t) = k'_\beta t^{-\lambda_\beta}$, and assuming just for simplicity $\lambda_\alpha = \lambda_\beta = \lambda_\mu$, the resulting differential equation with time-dependent coefficients is

$$\frac{d\mu}{dt} = -(k'_\alpha + k'_\beta)t^{-\lambda_\mu}\mu + k'_\alpha t^{\lambda_\mu}.$$

The general solution for rectangular voltage-clamp pulses is given by

$$\mu(t) = \mu_\infty + (\mu_0 - \mu_\infty)exp(-(t/\tau_\mu)^{1-\lambda_\mu})$$

with $\tau_\mu = (\frac{1-\lambda_\mu}{k'_\alpha + k'_\beta})^{\frac{1}{1-\lambda_\mu}}$ for all $0 < \lambda_\mu < 1$ and the classical Hodgkin-Huxley type solution is revealed by the limit $\lambda_\mu \to 0$. This fractal model provides an excellent fit for the decaying (inactivating) part of the Na-current in node of Ranvier of myelinated axons, without assuming more than one closed state for the k-system.

SODIUM CHANNELS III

716.1

MODULATION OF Na CURRENT DENSITY IN CLONAL PITUITARY CELLS BY CHRONIC TREATMENT WITH DIHYDROPYRIDINES. E. Monjaraz, U. Meza, A. Navarrete, and G. Cota*. Dept. of Physiology, Biophysics and Neurosciences, Cinvestav, Mexico, DF 07000.

Several previous observations from our laboratory in anterior pituitary cells raise the possibility that high threshold Ca channels may contribute to regulate the expression and/or function of Na channels. To test this possibility, pituitary GH3 cells were grown 4-5 days either under standard conditions or in the presence of 0.5 μM nimodipine or 0.5 μM BAY K 8644. After a recovery period of 60-90 min in the absence of dihydropyridines, whole-cell sodium currents were recorded by using the patch clamp technique. Sodium current density decreased to 50% of its control value in nimodipine-treated cells, and increased by a factor of 2.5 in cells exposed to BAY K 8644. Chronic treatment with these drugs did not modify the time course of the sodium current nor the voltage dependence of Na channel activation or inactivation. Furthermore, the stimulatory effect of BAK K 8644 on sodium current density was blocked by simultaneous incubation with cycloheximide or actinomycin D. The results indicate that calcium influx through L-type Ca channels has a positive influence on the long-term activity of Na channels.

716.2

ROLE OF PROTEIN KINASE C ISOFORMS IN THE REGULATION OF THE VOLTAGE-DEPENDENT SODIUM CHANNELS IN ADRENAL CHROMAFFIN CELLS. T. Yanagita, H. Kobayashi, R. Yamamoto, T. Yuhi, M. Urabe, H. Yokoo and A. Wada*. Miyazaki Medical College, Miyazaki 889-16, Japan.

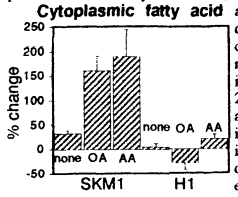
We examined whether protein kinase C (PKC) is involved in the regulation of Na channels in cultured bovine adrenal chromaffin cells. The chromaffin cells contained PKC α, ε and ζ isoforms, but not β, γ and δ isoforms. The immunoreactive PKC α and ε but not ζ were translocated from cytosol to membrane by a 15 h treatment of the cells with 12-O-tetradecanoylphorbol-13-acetate (TPA; 100 nM) and phorbol 12, 13-dibutyrate (PDBu; 100 nM), activators of PKC. The treatment decreased the B_{max} of [^{3}H]saxitoxin binding to the cells without altering the K_d value, and also reduced the veratridine-induced ^{22}Na influx, while 4 α-TPA, an inactive analogue, had no effect. The effect of TPA was abolished by H-7, an inhibitor of PKC. Even a 2 h pulse-treatment with PDBu followed by a 13 h incubation without PDBu caused the comparable decrease in [^{3}H]saxitoxin binding with the continuous 15 h treatment.

These results suggest that functional Na channels are down-regulated by a delayed-effect of short-term activation of PKC α and/or ε isoforms.

716.3

MODULATION BY INTRACELLULAR FATTY ACIDS OF HUMAN MUSCLE SODIUM CHANNELS EXPRESSED IN HEK293 CELLS IS DEPENDENT ON α SUBUNIT ISOFORM AND PATH OF DELIVERY. S. J. Wieland*[1], O-h. Gong[1], H. Poblete[1], J. E. Fletcher[2], L.-Q. Chen[3] and R. G. Kallen[3] 1. Dept. of Anat. & Neurobiology, 2. Dept of Anesthesiology, Med. Coll. of Penn. & Hahnemann Univ.; 3. Dept. of Biochem. & Biophysics, Univ. of Penn. School of Medicine

Free fatty acids (FFA) participate in signalling pathways, including the arachidonic acid (AA) cascades and activation of protein kinase C. These lipids are implicated in the direct and indirect modulation of a spectrum of voltage-gated ion channels. Skeletal muscle Na channels can be either activated or inhibited by FFA exposure; the response is dependent on both FA structure and site of exposure. Cytoplasmic delivery of 5 μM AA augmented the voltage-activated Na current of recombinant SkM1 skeletal muscle Na channels expressed in HEK293 cells by 190% (±54 SE, n=7) over a 20 min period. Similar results were seen with 5 μM oleic acid (OA). Na currents in cells expressing H1 cardiac muscle Na channels were insensitive to AA treatment (Figure 1), and exposure to OA inhibited the H1 currents over a 20 min period by 29% (±13 SE n=5). Increase in SkM1 current was not accompanied by shifts in voltage dependence of activation, steady-state inactivation or markedly altered kinetics of inactivation of the macroscopic current. Fatty-acid induced increase in Na currents was not dependent on protein kinase activity. In contrast, both isoforms were reversibly inhibited by external application of unsaturated FFA. Thus the differential effects of FFA on skeletal muscle Na channels first noted in cultured muscle cells can be reproduced by expressing recombinant Na channels in epithelial cells. We conclude that 1. SkM1 has 2 sites of response to FFA, one which produces augmentation of macroscopic currents with intracellular FFA, and a second which produces inhibition with extracellular FFA; 2. H1 has only one site, which produces inhibition with extracellular FFA. Supported by the MDA.



716.4

MECHANISM OF MODULATION OF THE ADULT SKELETAL MUSCLE SODIUM CHANNEL BY FATTY ACIDS. S. Bendahhou, T.R. Cummins‡, F.J. Sigworth‡ and W.S.Agnew*. Depts. of Physiology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, 21205 and ‡Interdepartmental Neuroscience Program, Yale University School of Medicine, New Haven, CT, 06510.

Unsaturated fatty acids, such as arachidonic acid, have been shown to affect the functional properties of Na channels from neuroblastoma cells (J. Physiol. Lond. 419:95-119, 1989), squid giant axons (J. Membrane Biol. 95:113-120, 1987), cultured human skeletal muscle cells (Am. J. Physiol. 263:C308-C312, 1992) and striatal neurons (Neuron 11:633-644, 1993).

We have investigated the effect of arachidonic (AA) and oleic (OA) acids on the adult rat skeletal muscle Na channel (μ1) stably expressed in HEK293 cells using both whole-cell and cell-attached configurations. External applications of 1-10 μM AA or OA produced 1) a decrease in peak current 2) a hyperpolarizing shift in the inactivation curve and 3) a substantial enhancement of the rate of the fast inactivation without alteration of slow inactivation. These effects were not prevented by inhibitors of protein kinase C, cyclooxygenase or lipoxygenase. Palmitic acid, a saturated fatty acid, had no effect on μ1 sodium currents.

In order to investigate the mechanism by which cis-unsaturated fatty acids affect the sodium channel, mutant channels with defective inactivation (F1304Q) were tested. The peak current was still inhibited and the hyperpolarizing shift was observed in the presence of AA. AA also decreased the current in cells where fast inactivation was removed with 100 μM chloramine-T. Preliminary results indicate that the gating current is also decreased by AA.

These data together suggest that the cis-unsaturated fatty acids may block the sodium channels directly from the extracellular side and/or through perturbations of the lipid environment. The mechanism underlying the effect of cis-unsaturated fatty acids may not involve the inactivation particle, at least not in the decrease of the peak amplitude. Because we have observed a decrease in both peak ionic and gating currents, we postulate that unsaturated fatty acids regulate electrical activity in the membrane by inducing the internalization of Na channels.

716.5

MODULATION OF SODIUM CHANNELS BY INSECT-SELECTIVE SCORPION AND SPIDER TOXINS.

T. M. Norris, D. Lee and M. E. Adams*. Depts. of Entomology and Neuroscience, University of California, Riverside, CA 92521.

A subset of arthropod Na^+ channel toxins are known to be selective for insect neurons. Two insect-selective toxins which show different types of Na^+ channel modulation are LqhαIT and μ-Aga-IV. Both toxins cause repetitive activity in insect motoneurons. LqhαIT increases both the amplitude and duration of evoked synaptic currents at the neuromuscular junction. In contrast, μ-Aga-IV produces a dramatic increase in spontaneous transmitter release.

Whole cell patch clamp recordings were performed to examine the effects of these toxins directly on sodium currents in insect neurons (*Heliothis virescens*). LqhαIT (200 nM) slows the rate of Na^+ channel inactivation and more than doubles peak current, effects which are consistent with an α-scorpion toxin-like action. Mammalian neurons (rat DRG) were markedly less sensitive to this toxin. μ-Aga-IV dramatically shifts the voltage dependence of Na^+ channel activation in insect neurons to more negative potentials and slows the rate of channel inactivation. These effects resemble in some respects the action of mammalian β-scorpion toxins. μ-Aga-IV showed no toxicity upon intracranial injection into mice and had no significant effect on rat DRG neuronal sodium currents. These data demonstrate that LqhαIT and μ-Aga-IV selectively modulate insect sodium channels in ways that explain their paralytic neuromuscular actions, and that the modulatory actions of these toxins resemble those of the mammalian α- and β-scorpion toxins, respectively.

716.6

AMINE-MODIFIED DERIVATIVES OF PD85639 AS SODIUM CHANNEL BLOCKERS. Sheryl J. Hays*, Ioannis Roufos and Roy D. Schwarz, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Co., Ann Arbor, MI. 48105.

Voltage-dependent sodium channels have been proposed as the site of action for anticonvulsant, antiarrhythmic, and local anesthetic agents. A structure-activity relationship study of a series of novel Na^+ channel blockers, structurally related to N-[3-(2,6-dimethyl-1-piperidyl)propyl]-α-benzene acetamide (PD85639), will be presented. The diphenylacetic acid portion of PD85639 was unchanged throughout the study, while structural features in the amine and amide portions of the molecule were altered. The compounds were tested for inhibition of veratridine-stimulated Na^+ influx in CHO cells expressing the alpha subunit of type IIA Na^+ channels. Our studies show that a three carbon spacer between the amine and the amide groups was optimal and a secondary amide linkage is preferred. Additionally, the presence of an aromatic ring in the vicinity of the amine group greatly enhanced the inhibitory activity of these derivatives.

716.7

EXTRACELLULAR APPLICATION OF QX-314 BLOCKS SODIUM CHANNELS AND CAUSES LOCAL ANESTHESIA. K.R. Bley*, M. Khabbaz, D.D. Blissard, C. Lee and J.C. Hunter, Depart. Analgesia, Roche Biosciences, Palo Alto, CA 94305.

QX-314, the quaternary derivative of lidocaine, is widely regarded to block Na^+ channels only following intracellular application, as its permanent positive charge is thought to restrict lipid bilayer permeation. Consequently, QX-314 and other permanently charged Na^+ channel blockers are not believed to be local anesthetics (see Hille, 1992, p. 405). In order to test these assumptions, we have examined the ability of QX-314 to block action potential propagation and cause local anesthesia. Reduction of nerve impulse conduction is proportional to the degree of Na^+ channel blockade. Accordingly, extracellular recordings were made of C-fiber compound action potentials in rat vagus nerves *in vitro*. Cumulative concentration-inhibition curves to superfused lidocaine or QX-314 were constructed using 10 min exposures to 1, 10, 100 and 1000 μM compound. Tonic block was measured with a slow rate of nerve stimulation (0.03 Hz), and phasic block with a 30 Hz burst of nerve stimulation at the end of each 10 min period. High frequency nerve stimulation shifted the IC_{50} for lidocaine only marginally, from 220 μM (tonic) to 210 μM (phasic). In contrast, the QX-314 IC_{50}s were >1000 μM (tonic) and 380 μM (phasic). Frequency-inhibition curves (0.1-30 Hz) were measured in the presence of ~IC_{50}s for tonic block. Lidocaine, a kinetically fast Na^+ channel blocker (τ_{rec} ~ 0.2 s in the heart), produced appreciable phasic block only at 30 Hz, whereas QX-314 (τ_{rec} ~ 300 s) caused significant phasic block even at 0.1 Hz. To measure local anesthesia, four intradermal injections of varying concentrations of lidocaine or QX-314 were made on the lower back of guinea-pigs. At 5, 10, 20 and 30 min after dosing, skin sensitivities were tested by pricking the skin with a needle. The number of negative responses (no skin twitch) out of five yielded EC_{50} values (expressed in %) of 0.116 for lidocaine and 0.235 for QX-314. The onset and duration of local anesthetic effects produced by QX-314 were slower and longer, respectively, compared to lidocaine. These studies suggest that QX-314 can penetrate cell membranes to block Na^+ channels. Penetration is slower than for lidocaine, but is still sufficiently fast to cause acute effects like local anesthesia.

716.8

CHICK EMBRYO SYMPATHETIC NEURONS: A MODEL TO STUDY SODIUM CHANNEL NEUROTOXINS. A.L. Rivera Rentas*, J.A. Mercado, [1]R. Rosa, [1]A. Rodríguez, [2]T.R. Tosteson, [2]I. González, [3]W. Silva, [4]L. Beress, G. Escalona de Motta. Inst. Neurobiology, Depts. of Biology, [1]Chemistry and [2]Marine Sciences, University of Puerto Rico, San Juan, P.R., [3]Pharmacology Dept., UCC, Bayamón, P.R., and [4]Toxicology Inst., Kiel University, Germany.

In this work we used primary cultures of chick embryo sympathetic neurons as a model system to study the mechanisms underlying the actions of known and new toxins on voltage sensitive sodium channels. Pharmacologically isolated inward sodium currents (I_{Na}) elicited by depolarizing stimuli were examined using the whole-cell technique in cultures after 48 hrs incubation. I_{Na} was described in terms of ionic nature, voltage dependence of activation and inactivation, and sensitivity to specific toxins. Tetrodotoxin and μ-conotoxin GIIIA blocked I_{Na} amplitude by nearly 80% and 20%, respectively. Venom samples of the scorpion *L. q. haebreus* inhibited inactivation at the μg/ml range and caused a delay in this proccess seen tail currents blockade at fg/ml. The site 5 toxin, brevetoxin type 1 (PbTx-1) from *P. brevis* shifted toward more negative potentials the voltage dependent processes and delayed I_{Na} reactivation. In other experiments the actions two novel agents were examined. An alkaloid from the sponge *A. oroides*, Dibromosceptrin, displaced ^{3}H-saxitoxin in rat brain synaptosomes, and as expected for a site 1 toxin, decreased by nearly 40% I_{Na} amplitude. It also shifted toward more negative potentials the voltage dependence of inactivation. A toxic extract from the dinoflagellate *O. lenticularis*, did not affect ^{3}H-PbTx3 binding, decreased I_{Na} amplitude by 20% and shifted by 30 mV to more negative potentials the voltage dependence of inactivation. Different from the brevetoxins reactivation were not altered by this dinoflagellate extract. (Supported by GM08102 & NIH48190)

716.9

INTERACTION OF BESIPIRDINE WITH THE VOLTAGE DEPENDENT SODIUM CHANNEL. L. Tang*, C.P. Smith, F.P. Huger and S. Kongsamut
Neuroscience Product Group Unit, Hoechst Roussel Pharmaceuticals, Inc., P.O. Box 2500, Somerville, NJ 08876.

The alkaloid toxins veratridine and batrachotoxin bind to a site (so-called site II) on the voltage dependent sodium channel where they cause persistent activation of the channel and depolarize the cell. Tetrodotoxin and saxitoxin bind to a different site on the sodium channel and can block the effects of veratridine and batrachotoxin. Other known modulators of sodium channels include anticonvulsants and local anesthetics which inhibit sodium currents and displace [^{3}H]batrachotoxin binding. Besipirdine (HP 749) is a compound undergoing clinical trials for efficacy in treating Alzheimer's Disease. Among other pharmacological effects, besipirdine inhibits voltage dependent sodium and potassium channels. We present here a more detailed study on the interaction of besipirdine with voltage dependent sodium channels. Besipirdin displaced [^{3}H]batrachotoxin binding with an IC_{50} of 5.5±0.2 μM in a rat brain vesicular preparation and inhibited veratridine-stimulated increases in intracellular free sodium ($[Na^+]_i$) and calcium ($[Ca^{2+}]_i$) in primary cultured rat cortical neurons in a concentration dependent manner. Furthermore, besipirdine dose-dependently inhibited veratridine-stimulated release of [^{3}H]norepinephrine (NE) from rat cortical slices. When examined in greater detail, besipirdine was found to inhibit [^{3}H]batrachotoxin binding in vesicular membranes competitively. However, when examined in rat brain synaptosomes, the antagonism by besipirdine was found to be non-competitive; that is, the maximal stimulation of $[Ca^{2+}]_i$ induced by veratridine decreased with increasing concentration of besipirdine. These results are discussed in relation to possible anticonvulsant activity of besipirdine.

716.10

ACTIONS OF PYRETHROID INSECTICIDES ON SODIUM CHANNELS EXPRESSED IN *XENOPUS* OOCYTES. Timothy J. Smith* and David M. Soderlund. Field of Environmental Toxicology and Dept. of Entomology, New York State Agric. Expt. Station, Cornell Univ., Geneva, NY 14456.

Pyrethroids make up a large class of potent neurotoxic insecticides whose principal target is the voltage-gated sodium channel. In intact nerves, pyrethroids induce prolonged sodium currents and afterpotentials which lead to uncontrolled repetitive firing or use-dependent nerve block. To further explore the molecular basis of pyrethroid action on sodium channels we employed the heterologous expression of sodium channel subunits in *Xenopus* oocytes. The rat brain IIa sodium channel α subunit and the rat brain β_1 subunit mRNAs were synthesized *in vitro* from cloned cDNAs. The α subunit mRNA was injected into oocytes either alone or in combination with the β_1 subunit mRNA and the actions of the pyrethroid deltamethrin on expressed sodium currents were assessed by two-electrode voltage clamp. Deltamethrin prolonged the time course of sodium channel inactivation and produced tail currents following repolarization that were qualitatively similar to the effects produced on sodium channels in intact nerves. The presence of the β_1 subunit enhanced rapid inactivation, as anticipated, but did not modify the effects of deltamethrin. These findings imply that the binding domain involved in the neurotoxic action of pyrethroids is associated with the sodium channel α subunit. Other pyrethroids also prolonged sodium channel inactivation and produced sodium tail currents in this assay system. The rate of tail current decay in this system was found to be compound-dependent.

716.11

VOLTAGE-DEPENDENT ACTIVATION AND LIDOCAINE (LI) SENSITIVITY OF BRAIN VOLTAGE-DEPENDENT SODIUM CHANNELS (VDSC) DURING DEVELOPMENT. C. Castillo, M.E. Díaz, B.C. Salazar, Villegas,G.M.*, E. Recio-Pinto. Instituto Internacional de Estudios Avanzados (IDEA) Apartado 17606 Caracas 1015-A, Venezuela. Anesthesiology Dept. Cornell University Medical College. New York, NY 10021.

In the brain, the number and types of VDSC change[1,2] and their TTX/STX affinity increase during development[2]. We are investigating whether other properties of VDSC undergo changes during forebrain development. Sodium channel activity from newborn (P0) and postnatal day 15 (P15) rat brains were studied in planar lipid bilayers in the presence of BTX and in symmetrical 200 mM NaCl. The steady state activation curve showed a hyperpolarization shift during development: the midpoint potential values were -54mV and -74mV for channels derived from P0 and P15 forebrains respectively. The maximal fractional open time was lower in channels derived from P0 than P15, due to a higher proportion of short- and long-lived channel closures in channels from P0. The level of LI-induced open channel block did not change during development. LI blocked the open state of brain channels with about ten times higher potency than that of muscle derived channels[3]. The higher LI affinity of brain channels was mainly due to a lower k_{off} value, indicating that the conformation of the channel structures defining the LI binding site is not identical in brain and muscle-derived sodium channels; while they appear to be the same in the various brain sodium channels. [1]Sheinman et al.,1989;[2]Villegas et al., 1994; [3]Zamponi et al., 1993.

716.12

SELECTIVE BLOCK OF TETRAMETHRIN-MODIFIED SODIUM CURRENT BY VITAMIN E. J-H. Song* and T. Narahashi. Dept. of Mol. Pharmacol. & Biol. Chem., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Pyrethroids are neurotoxic insecticides, which exert their effects by prolonging the open time of individual neuronal sodium channels. Occupational exposure to pyrethroids frequently leads to abnormal skin sensation or paresthesia. Vitamin E, (±)-α-tocopherol, has been known to reduce the cutaneous paresthesia, but the mechanism of action remains to be seen. Since the sodium channel is the major target site of pyrethroids, it is possible that vitamin E interacts with pyrethroids at their receptor site on the sodium channel. In present patch clamp study, we found the selective block of tetramethrin (a type I pyrethroid)-modified sodium current by vitamin E. Vitamin E 10 μM and 30 μM blocked 10 μM tetramethrin-modified tetrodotoxin-sensitive (TTX-S) sodium currents in rat cerebellar Purkinje cells by 31 % and 77 %, respectively, and in rat dorsal root ganglion cells by 34 % and 76 %, respectively, with no effect on the tetramethrin-unmodified current. The concentration-response curve for tetramethrin effects was shifted to higher concentrations by vitamin E in a competitive manner. Repetitive discharges and elevated depolarizing after-potential caused by tetramethrin were effectively blocked by vitamin E. Tetramethrin-unmodified tetrodotoxin-resistant (TTX-R) sodium current in dorsal root ganglion cell was blocked by vitamin E. Vitamin E block of tetramethrin-modified TTX-S sodium current was not dependent on voltage or depolarizing duration. Vitamin E could not reverse the tetramethrin-induced shift in the current-voltage curve, but partially reversed the shift in the steady-state sodium channel inactivation curve of TTX-S sodium channels. Vitamin A and its metabolic derivative, retinoic acid, slightly reduced the tetramethrin-modified sodium current accompanied by the block of normal sodium current. The selective block of tetramethrin-modified sodium current by vitamin E seems to be responsible for vitamin E alleviation of paresthesia.

716.13

δ-CONOTOXINS, A FAMILY OF SUBTYPE-SPECIFIC *CONUS* PEPTIDES WHICH INHIBIT INACTIVATION OF VOLTAGE-SENSITIVE SODIUM CHANNELS. K. Shon[1], M.M. Grilley[1], H. Terlau[2], W. Stühmer[2], W.R. Gray[1], J.S. Imperial[1]* and B.M. Olivera[1]. Dept. of Biology[1], Univ. of Utah, Salt Lake City, UT 84112; Max-Planck-Institut für experimentelle Medizin[2], D-37075 Göttingen, Germany.

A vertebrate-specific δ-conotoxin, δ-PVIA (the "lock-jaw peptide") was purified and characterized from the venom of *Conus purpurascens*, a fish-hunting cone snail (Shon et al., 1995, *Biochemistry* 34, 4913). The two δ-conotoxins previously characterized, δ-TXVIA (the King-Kong peptide") and δ-GMVIA (Hillyard et al., 1989, *Biochemistry* 28, 358; Shon et al. *Biochemistry*, 1994, 33, 11420) were from mollusc-hunting cone snail venoms, and exhibited potent activity on molluscan voltage-sensitive sodium channels. Like the other δ-conotoxins, δ-PVIA is a very hydrophobic peptide, with 29 amino acids and 3 disulfide bridges. Although the disulfide bridge framework is conserved in all three δ-conotoxins, the non-cysteine amino acids are not conserved in the three family members. δ-Conotoxin PVIA elicits excitatory symptoms in mice and fish, but is inactive in molluscs even at doses 100-fold higher. The peptide inhibits sodium channel inactivation in both cloned mammalian sodium channels expressed in *Xenopus* oocytes, as well as in cultured mammalian neurons. The δ-conotoxins are a promising group of *Conus* peptides for discriminating between various vertebrate subtypes of voltage-sensitive sodium channels. δ-Conotoxin TXVIA is generally inactive in vertebrate systems; δ-conotoxin GMVIA causes excitatory symptoms but only in immature mammals. In contrast, δ-conotoxin PVIA exhibits broad activity in a wide range of vertebrate systems tested. cDNA clones for all three δ-conotoxin sequences have been characterized; this makes it possible to systematically identify additional δ-conotoxins from *Conus* species using molecular biological techniques.

716.14

NEW CONOTOXINS WHICH BLOCK NEURONAL SODIUM CHANNELS. J.M. McIntosh[1,3], A. Hasson[4], M.E. Spira[4], W.R. Gray[1], W. Li[1], M. Marsh[2], D.R. Hillyard[2], H. Terlau[5], W. Stühmer[5] and B.M. Olivera.[1]* Depts. of Biology[1], Pathology[2] and Psychiatry[3], Univ. of Utah, Salt Lake City, Utah, 84112; Dept. of Neurobiology[4], Inst. of Life Sciences, Hebrew Univ. of Jerusalem, Jerusalem, 91904 Israel; Max-Planck-Institut für experimentelle Medizin[5], D-37075 Göttingen, Germany.

We previously characterized ω-conotoxins, μ-conotoxins and δ-conotoxins, which target voltage-sensitive calcium channels, voltage-sensitive sodium channels and inhibit sodium channel inactivation, respectively. We report a new family of peptides, the μO-conotoxins. The 31-residue peptides, μO-conotoxins MRVIA/B, were purified from the venom of *C. marmoreus*. Their structures were confirmed by mass spectrometry, molecular cloning, and chemical synthesis. The peptides were isolated by electrophysiologically screening venom fractions in cultured *Aplysia* neurons, where these ligands potently and selectively block sodium conductance. μO-Conotoxins are also active in vertebrate systems; unlike the μ-conotoxins which are specific for muscle Na channels in adult rats, μO-conotoxins potently inhibit neuronal subtypes. Although the μO-conotoxins block voltage-sensitive sodium channels like the μ-conotoxins, the disulfide framework of the μO-conotoxins is that of the ω- and δ-conotoxins but not of the μ-conotoxins. Thus, together the ω-, δ- and μO-conotoxins define a superfamily of *Conus* peptides.

716.15

BRAIN VOLTAGE-DEPENDENT SODIUM CHANNELS SHOW TEMPORAL CHANGES IN THEIR LIDOCAINE APPARENT BINDING AFFINITY. B.C. Salazar, C. Castillo, M.E. Díaz, E. Recio-Pinto*. Anesthesiology Dept, Cornell University Medical College, New York, NY 10021. Instituto Internacional de Estudios Avanzados (IDEA), Apartado 17606, Caracas 1015-A, Venezuela.

Local anesthetics block the open state of various voltage-dependent sodium channels. In muscle-derived channels, lidocaine (LI) and the permanently charged lidocaine analog QX-314 produce a constant level of open channel block. In brain-derived channels, however, we found that when studied under identical conditions, the level of open channel block induced by LI and QX-314 showed periods in which it was highly reduced. Therefore, in brain channels, the level of open channel block shows two dominant modes, a high- and a low-affinity mode. The amount of time the channel expends in the low-affinity mode was larger in the presence of QX-314 than of LI. With QX-314 it was possible to detect the presence of more than one high-affinity mode in brain sodium channels. The various affinity modes suggest the presence of various open channel conformations. The level of the LI/QX-314-induced open channel block increased with depolarization. The magnitude of this voltage dependence was the same in the various LI/QX-314 affinity modes. This indicates that the distance between the lidocaine binding site and the intracellular channel aspect does not change with changes in the open channel conformation. Noise analysis showed that the various anesthetic affinity modes display the same k_{off} but different k_{on} rates, indicating that the channel open conformational changes most likely involve changes in the hydrophilic pathway leading to the binding site, rather than changes at the binding site itself.

716.16

INHIBITION OF VERATRIDINE-INDUCED [14C]-GUANIDINE FLUX IN CNAIIA-1 CELLS. S. Connaughton, T. Wolcott, L.D. Margolin & J.B. Fischer*, Cambridge Neuroscience, Inc., Cambridge, MA 02139.

Experiments were performed in a Chinese hamster ovary cell line that stably expresses the alpha-1 subunit of the mammalian type IIA neuronal sodium channel (CNAIIA-1). Electrophysiological studies demonstrate that CNAIIA-1 cells generate a rapidly inactivating, inward sodium current with normal time course and voltage dependence (Scheuer et al., 1990; Knapp et al., 1993).

The neurotoxin,veratridine, causes persistent activation of sodium channels at resting membrane potential by blocking inactivation and this effect is enhanced by scorpion venom. Veratridine-induced [^{14}C]-guanidine flux in CNAIIA-1 cells in a dose dependent manner with maximal flux occuring at 125-300 µM. Scorpion toxin (6 µg/ml) potentiated this flux up to 14-fold. Veratridine-induced flux in the absence and presence of scorpion toxin (6 µg/ml) was linear with time up to at least 60 min and 45 min, respectively. TTX inhibited the veratridine (90 µM)-induced flux in the absence and presence of Scorpion toxin (6 µg/ml) with an IC_{50} of 6.8 and 33.8 nM, respectively. Several anticonvulsants, the sodium channel blocker, BW 1003C87 and a number of our putative sodium channel blockers inhibited veratridine induced [^{14}C]-guanidine flux as shown below:

COMPOUND	IC50 ± S.E.M.(µM)	COMPOUND	IC50 ± S.E.M.(µM)
LAMOTRIGINE	44.4 ± 14.4, n=5	PHENYTOIN	32.6 ± 0.96, n=2
BW 1003C87	2.21 ± 0.38, n=2	CNS 1237	1.64 ± 0.04, n=5
RILUZOLE	2.15 ± 0.96, n=4	CNS 1145	2.03 ± 0.33 n=7

In conclusion, this flux assay in CNAIIA-1 cells is a useful high throughput screen for compounds with putative type IIA sodium channel blocking activity.

SODIUM CHANNELS IV

717.1

STABLE COEXPRESSION OF α and β1 SODIUM CHANNEL SUBUNITS IN CHO-K1 CELLS: EFFECT OF THE β1 SUBUNIT ON THE DENSITY AND NEUROTOXIN BINDING PROPERTIES OF THE EXPRESSED CHANNELS. D.W. Bonhaus*, R.C. Herman, C.M. Brown, Z. Cao, L.-F. Chang, D.N. Loury, P. Sze, L. Zhang and J. C. Hunter Syntex Research, Palo Alto, CA 94034 and Edinburgh Scotland.

Mammalian brain sodium channels consist of a principal α subunit and two smaller β subunits. The α subunit, by itself, forms a functional channel, whereas the β1 subunit may modify the stability and inactivation kinetics of α subunit. To examine the effect of the β1 subunit on the functional expression of α subunit sodium channels, a Chinese hamster ovary (CHO) cell line stably coexpressing the rat IIA α and human β1 subunits was generated. Cells expressing both the α and β1 subunits had a five fold higher density of [^{3}H]Saxitoxin binding sites and a 2 fold greater maximum rate of veratridine-stimulated [^{14}C]guanidinium influx than did cells expressing only the α subunit. Brevetoxin, α Scorpion toxin, and the pyrethroid RU39568 increased (5-20 fold) veratridine-stimulated [^{14}C]guanidinium ion influx in both cell lines with similar EC_{50} values. However, these neurotoxins stimulated [^{3}H]Batrachotoxinin ([^{3}H]BTX) binding only in membranes of cells expressing both α and β1 subunits; brevetoxin, α scorpion toxin and RU39568 had no effect on [^{3}H]BTX binding to membranes of cells expressing only the α subunit. These findings indicate that coexpression of the β1 subunit increased the number of functional sodium channels and enabled the neurotoxins to evoke conformational changes in the α subunit associated with an increase in [^{3}H]BTX binding. These findings are consistent with a role for the β1 subunit in maintaining the α subunit in the membrane in a functional, neurotoxin-sensitive, conformation.

717.2

PRIMARY STRUCTURE AND FUNCTIONAL EXPRESSION OF THE β2 SUBUNIT OF THE RAT BRAIN NA^+ CHANNEL. L.L. Isom*[1], D.S. Ragsdale[2], K.S. DeJongh[3], B.F.X. Reber[4], and W.A. Catterall[2]. [1]Dept. of Pharmacology, The Univ. of MI, Ann Arbor, MI 48109, [2]Dept. of Pharmacology, Univ. of WA, Seattle, WA 98195, [3]CTI Seattle, WA 98119, [4]Dept. of Pharmacology, Univ. of Berne, Switzerland.

Voltage-sensitive Na^+ channels are the membrane proteins responsible for the rising phase of the action potential observed in excitable cells. Na^+ channels isolated from rat brain are a heterotrimeric complex composed of an α subunit and two auxiliary β subunits, β1 and β2. β1 has been shown to be non-covalently associated with α while β2 is covalently linked to α by disulfide bonds. The β1 subunit has been cloned, sequenced and expressed and shown to play a modulatory role in sodium channel kinetics and expression levels. We now describe the primary structure of the rat brain Na^+ channel β2 subunit. The deduced primary structure of β2 shows that it is a 20,902-dalton protein that contains a cleaved signal peptide, a single putative transmembrane domain, and 4 potential N-linked glycosylation sites. β1 and β2 subunits share topological similarities, yet are not homologous at the nucleotide or amino acid levels. Comparison of the β2 peptide sequence with other known sequences in the peptide databases shows that β2 contains a domain that is homologous to a neuronal cell adhesion protein that has been implicated to play a role in the formation of the developing nervous system. Northern blot analysis of mRNA expression suggets that β2 is limited to the central nervous system. Coexpression of Type IIa α and β2 subunits in *Xenopus* oocytes results in a β2 concentration-dependent increase in peak current amplitude, an increase in the rate of macroscopic inactivation, and a small hyperpolarizing shift in the voltage-dependence of inactivation. These functional effects of β2 appear to be additive with β1. Thus, β2 subunits may function as important regulators of Na^+ channel expression, modulating electrical excitability in the embryonic and adult central nervous systems.

717.3

Functional effects of neuronal Na^+ channel β1-subunit heterologously co-expressed with µ1 Na^+ channel in Xenopus oocytes I.F. Potts* & W.S. Agnew Dept. of Physiology, Johns Hopkins School of Medicine, Baltimore, MD 21205.

The mammalian adult skeletal muscle voltage-sensitive Na^+ channel underlies the action potentials observed in the sarcolemma. Functional µ1 channels can be heterologously expressed in *Xenopus* oocytes using RNA encoding the α-subunit alone but display anomalously slow inactivation kinetics (gating mode 2) unless co-expressed with neuronal β_1-subunit RNA, suggesting a modulatory role for that auxiliary subunit (which is closely related or identical to the skeletal muscle Na^+ channel β-subunit). To test whether the β_1-subunit might also enhance the surface expression of functional µ1 channels, peak I_{Na} was observed over time in *Xenopus* oocytes heterologously expressing µ1 alone or with the β_1-subunit. Maximal peak expression of Na^+ current occurred at least two times faster in the presence of the β_1-subunit than when µ1 was expressed alone. To test whether the β_1-induced stabilization of mode 1 gating conformation requires co-translation of the two subunits in *Xenopus* oocytes, RNA injections were delayed by 24 or 48 hours. Regardless of subunit RNA injection order or the length of delay between injections (≤48 hours) all cells receiving β_1-subunit RNA exhibited significantly greater I_{fast}/I_{slow} ratios than cells injected only with RNA encoding µ1 α-subunit. "post-injected" β_1 induced a significant decrease in slow-inactivating (mode 2) whole cell I_{Na} with no net change in channel density (as estimated from weighted whole cell peak I_{Na}) it seems likely that the observed shift in gating mode prevalence represents a conversion of channels already in the plasma membrane from mode 2 to mode 1 conformation.

717.4

GENES ENCODING THE β1 SUBUNIT OF VOLTAGE-DEPENDENT BRAIN SODIUM CHANNEL IN RAT, MOUSE AND HUMAN CONTAIN AN INTRON. Sulayman D. Dib-Haji* and Stephen G. Waxman. Department of Neurology, Yale University School of Medicine, New Haven, CT and Neuroscience & Regeneration Research Center, PVA/EPVA, VA Medical Center, West Haven, CT 06516.

In an effort to understand the fuctional role of the different subunits of voltage-dependent sodium channels, we recently described a transcript (β1.2) that is closely related to the voltage-dependent brain sodium channel β1 transcript (β1.1). cDNAs of the β1.2 transcript were analyzed and revealed two major differences between β1.1 and β1.2. First, an 86 bp insert in β1.2 is present in the 3' untranslated sequence, 6 bp downstream of the translation termination codon. Second, a single nucleotide substitution changes the Aspargine at position 110 to Serine; this change eliminates a putative N-linked glycosylation site that is present in the β1.1 proteins of rat and human channels. These observations suggested that this new transcript could represent an isoform (β1.2) of the previously described β1.1 species.

We now report that the 86 bp insertion has the hallmarks of an intron. The 5' and 3' termini of the insert are the invariant dinucleotides GT and AG, respectively. A polypyrimidine-rich sequence precedes the AG dinucleotide and the sequence C T A A G, located 26 bp upstream of the 3' end is a good match to branch point motif C/T T A/G A C/T (the highlighted A is the branch nucleotide) of higher eukaryotic introns. Using PCR, we have amplified segments of rat, mouse and human genomic DNAs that contain this intron. Preliminary evidence suggests that the intron is conserved in the three species. We will utilize this intron to determine whether a single β1 gene is present in the three genomes.

Ths work is supported by funds from the VA and NMSS.

717.5

SODIUM CHANNEL β1 SUBUNIT mRNA IN OLFACTORY SYSTEM DURING POSTNATAL DEVELOPMENT AND FOLLOWING DENERVATION. S.Sashihara*, C.A.Greer, Y.Oh and S.G.Waxman. Depts. of Neurol. and Neurosurg. Yale Univ., New Haven, CT 06510 & PVA/EPVA Neurosci. and Regen. Cnt., VAMC, W. Haven, CT 06516.

We investigated the developmental and denervation-induced regulation of the β1 subunit (Naβ1) of the Na^+ channel in rat olfactory system. *In situ* hybridization at postnatal days 4, 7, 14, 28 and 47 shows that Naβ1 mRNA expression is upregulated with increasing age, but with different time courses in mitral, tufted, and pyramidal cells. In mitral cells, label was detected at postnatal day 4 (P4) and gradually increased to peak at P14. Tufted cells were devoid of Naβ1 mRNA at P4 and P7, but at P14 through adult, most tufted cells expressed Naβ1 mRNA. In pyramidal cells of pyriform cortex (PC), Naβ1 expression is further delayed, and is not clearly detectable until P14, with peak expression at P28.

We surgically deafferentated the olfactory bulb (OB) at P30, and compared effects of such deafferentation on Naβ1 with those for Na^+ channel α subunit (Naα) mRNAs. Within 5 days of surgery, the signals for Naβ1 and NaαII expression in mitral cells were decreased to low-to-moderate levels. In pyramidal cells, Naβ1 mRNA expression was moderately decreased without significant change in NaαII mRNA. The deafferentation had no apparent effects on expression of mRNAs αI and αIII in these cells of the OB and PC. These data indicate that Naβ1 mRNA is differentially expressed in various cell types in the olfactory system during normal development. Moreover, deafferentation data suggest that the expression of Naβ1 is regulated independently of Naα mRNAs via cell-specific and pathway-specific mechanisms. Supported in part by the VA, NMSS and NIH.

717.6

DIFFERENTIAL Na^+ CHANNEL β1 SUBUNIT mRNA EXPRESSION IN DRG NEURONS IN VITRO AND IN VIVO. Y. Oh*, S. Sashihara+, K. McNabola+, J.A. Black+, S.G. Waxman+. *Department of Medicine, Division of Nephrology, and Neurobiology Research Center, University of Alabama, Birmingham, AL 35294; and +Department of Neurology, Yale University School of Medicine, New Haven, CT 06510.

The voltage-sensitive Na^+ channel β1 subunit (Naβ1) mRNA has recently been localized within the developing and adult CNS, where its expression is regulated temporally and spatially. In the present study, we have examined the expression of Naβ1 mRNA in adult rat dorsal root ganglion (DRG) neurons *in vitro* and *in vivo* using non-radioactive *in situ* hybridization cytochemistry. At 3-4 days in vitro, Naβ1 mRNA was prominent in DRG neurons. The level of Naβ1 expression appeared to be greater in medium-to-large (>25 μm in diameter) DRG neurons than in small (<25 μm) DRG neurons. The Naβ1 mRNA was localized to the somata of the DRG neurons and was not detectable in the neurities. In intact DRGs, results were consistent with those of *in vitro* studies. The level of Naβ1 mRNA expression was higher in intermediate-to-large (>25 μm) DRG neurons compared to that in small (<25 μm) DRG neurons. This cell body size-related Naβ1 mRNA expression is consistently observed beginning at postnatal day 4 and continues throughout development to adulthood. These results demonstrate that Naβ1 mRNA is expressed in PNS neurons *in vitro* and *in vivo*, and suggest that Naβ1 gene expression in DRG neurons is differentially regulated in relation to their cell body size. [Supported in part by the VA and NMSS]

717.7

THE *tipE* GENE OF *DROSOPHILA MELANOGASTER* ENCODES A NOVEL MEMBRANE PROTEIN REQUIRED FOR EXPRESSION OF *para* SODIUM CHANNELS IN XENOPUS OOCYTES. Linda M. Hall*, Guoping Feng, and Maninder Chopra. Department of Biochemical Pharmacology, SUNY at Buffalo, Buffalo, NY 14260.

Mutations in the *tipE* gene cause a temperature-induced paralysis and reduction in sodium channels as measured electrophysiologically (O'Dowd and Aldrich, *J. Neurosci.* **8**:3633, 1988) and by saxitoxin binding. Cloning by chromosome walking and transformation rescue has shown that the *tipE* gene encodes a novel 50,000 dalton protein. *In vitro* translation in the presence of microsomes shows that *tipE* is a glycosylated, integral membrane protein with two transmembrane domains. Both the amino and carboxy termini are on the cytoplasmic side of the membrane. The *tipE* protein is required in mid to late pupal development when the nervous system is undergoing final maturation in order to rescue paralysis in adults. In addition, expression is also required in adults to prevent a heat-induced lethality which is independent of the paralysis phenotype. Coexpression in Xenopus oocytes with the sodium channel α subunit encoded by the *para* locus shows that *tipE* is required for functional expression of this sodium channel. A dendrogram comparing previously cloned sodium channel α subunits from a variety of species suggests that many of those which have been difficult to express may require a *tipE* homologue. Site directed mutagenesis coupled with expression in Xenopus oocytes is being used to define functionally important domains on the *tipE* protein. [*para* clone provided by J. Warmke, P. Wang, L. Van der Ploeg (Merck) & R. Reenan, B. Ganetzky (U. Wisconsin)]

717.8

THREE NEW PUTATIVE VOLTAGE GATED SODIUM CHANNELS (VGSC) FROM HUMAN NEUROBLASTOMA (SH-SY5Y) CELLS: A PCR BASED CLONING STRATEGY. **J.L. Walewski*, J. Ziauddin, and E. Recio-Pinto** Depts. of Anesthesiology and Physiology, Cornell University Medical College, New York, N. Y. 10021

When exposed to retinoic acid, SH-SY5Y cells undergo neuronal differentiation, i.e., neurite outgrowth and the induction of electrical excitability (increased expression of voltage gated Na^+ and Ca^{2+} channels). To identify VGSC expressed by the undifferentiated or differentiated cells, total RNA from each treatment was transcribed into first strand cDNA, then subjected to two rounds of PCR (2nd nested), using degenerate primers specific to segments of the highly conserved third and fourth transmembrane regions of the alpha subunit. Cloning, restriction analysis, and sequencing revealed three distinct sub-clones (one from undifferentiated and two from differentiated cells) which are unique, yet highly homologous to other members of the VGSC family. Amino acid (AA) translation of the amplified cDNA demonstrated the expected high homology (Table 1) at the transmembrane regions, the presence of the IFM fast inactivation motif, and otherwise considerable AA variation in the III-IV cytoplasmic loop.

Table1

	HuVGSC	Hu Card	Rat II	Eel	Drosph	Jellyfish
CLONE	% Iden	% Iden	% Iden	% Iden	% Iden	% Iden
3	89	82	89	64	50	35
5	96	83	96	68	51	35
6	75	68	75	59	75	35

JLW is an Aaron Diamond Post-doctoral fellow, and this project is supported in part by the Aaron Diamond Foundation. Additional support was provided by the Dept. of Anesthesiology, CUMC.

717.9

Identification of a partial cDNA encoding a putative voltage-gated sodium channel from the parasitic flatworm *Bdelloura candida*. **Michael C. Jeziorski*, Robert M. Greenberg and Peter A.V. Anderson. The Whitney Laboratory, University of Florida, St. Augustine, FL.**

Alterations in the fast inward sodium current responsible for the initiation of action potentials in human muscle and heart have been implicated in the pathology of several disorders, such as paramyotonia congenita (PC) and long QT syndrome. The specific defect in these diseases, supported by identification of several mutations, is thought to be a decrease in the efficiency of transition from the open state to the inactivated state of the voltage-gated sodium channel (VGSC), resulting in a sustained inward current, depolarization of the membrane and loss of membrane excitability. The fast inward sodium current expressed by neurons of the platyhelminth *B. candida* also exhibits a non-inactivating, tetrodotoxin-sensitive component. We have used RT-PCR with degenerate primers to isolate a cDNA fragment from *Bdelloura* RNA that displays homology to known VGSCs. This fragment, which hybridizes to an RNA of approximately 5.5 kb, includes a sequence encoding the III-IV interdomain cytoplasmic loop, a region known to be one of several components essential to inactivation of VGSCs. This region of the *Bdelloura* channel contains an alanine in place of a threonine that is conserved in every other identified VGSC cDNA (T1491 in the rat brain II channel), and that is mutated in one manifestation of PC. Additional residues within the III-IV loop vary from other known sequences and may also underlie incomplete inactivation of the sodium current. Further work will be directed toward identification of the complete cDNA sequence, heterologous expression of the channel, and characterization of its sensitivity to tetrodotoxin. (Supported by NSF grant IBN-9410565 to PAVA and NRSA grant MH 10625 to MJ.)

717.10

EXPRESSION AND FUNCTION OF SPECIFIC SODIUM CHANNEL ISOFORMS IN EMBRYONIC NEURONS. D.D.Hodges[1], S. Takamatsu, B. McNevin, and D.K. O'Dowd*[1,2]. Dept. of Anat. & Neurobiol.[1] and Dev. & Cell Biol. [1,2] U. C. Irvine, CA 92717.

Voltage gated sodium channels play a key role in electrical excitability of cells in vertebrate and invertebrate nervous systems. The *para* gene encodes functional voltage-gated Na^+ channels in cultured *Drosophila* embryonic neurons. Using a combination of electrophysiology and single cell RT-PCR, we have previously shown that *para* transcripts containing alternative exons i and /or a are positively correlated with large I_{Na} density. We have initiated experiments to test the hypothesis that phosphorylation at a PKA site in exon a regulates I_{Na} density. Treatment of cultured neurons with forskolin, an activator of PKA, results in a 10-30% decrease in peak I_{Na}. The ability of other phosphorylation activators and inhibitors to modulate sodium currents is currently under investigation. We have also generated transgenic flies which express heat shock inducible ribozymes designed to eliminate all *para* transcripts or specifically those containing exon a. A preliminary hatching assay on one of these lines indicates that heat shock prior to *para* expression results in a small decrease in hatching and larvae that hatch display a sluggish phenotype similar to that described for *para* nulls. Other paralytic assays and RT-PCR analysis of total *para* mRNA from different developmental stages, as well as electrophysiological and single cell RT-PCR analysis of cultured embryonic neurons from these transgenic lines are in progress. These studies are supported by NIH grants NS27501 to DOD and NS07351 to DDH.

717.11

CHARACTERIZATION OF SODIUM CHANNEL GENES FROM INSECTICIDE-SUSCEPTIBLE AND INSECTICIDE-RESISTANT HOUSE FLIES. Patricia J. Ingles, Pamela M. Adams, Douglas C. Knipple and David M. Soderlund*. Dept. of Entomology, New York State Agric. Expt. Station, Cornell Univ., Geneva, NY 14456.

The *kdr* insecticide resistance trait of the house fly (*Musca domestica*), which confers reduced neuronal sensitivity to DDT and pyrethroid insecticides, exhibits tight genetic linkage to RFLP markers lying within a sodium channel gene that is homologous to the *para* gene of *Drosophila melanogaster*. The coding sequence of the house fly *para* gene was determined by direct automated DNA sequencing of PCR fragments obtained by amplification on first strand cDNA from adult heads. The predicted house fly amino acid sequence was >90% identical to that of the *D. melanogaster para* gene product. In contrast to the multiplicity of sodium channel isoforms that result from alternative exon usage in *D. melanogaster*, there was no evidence for the existence of multiple splice variants among the cDNAs obtained from adult house fly head preparations. The house fly *para* sequence identified in this study was most similar to the [$a^+b^-c^-d^+e^-f^-$] splice variant of *D. melanogaster*, which occurs as a minor variant in adults but not embryos. However, the house fly sequence also contained segments located within the region of anticipated alternative splicing that do not correspond to any of the identified alternative exons from *D. melanogaster*. Comparison of *para* coding sequences for the NAIDM (susceptible) and 538ge (*kdr*-resistant) house fly strains revealed several nucleotide substitutions that result in changes in amino acid sequence. The role of these sequence polymorphisms in conferring the *kdr* insecticide resistance phenotype remains to be determined.

717.12

CLONING OF A SODIUM CHANNEL α-SUBUNIT (PN1) FROM RAT DORSAL ROOT GANGLIA. L.M. Fish, L. Sangameswaran, S.G. Delgado, B.D. Koch, L.B. Jakeman, J.Kwan, and R.C.Herman*. Biotechnology Unit and Center for Peripheral Nervous System Res.,Syntex, Palo Alto, CA 94304.

We have isolated a full-length sodium channel α-subunit clone, designated peripheral sodium channel 1 (PN1), from a rat dorsal root ganglia (DRG) cDNA library. The cDNA encodes an open reading frame of 1984 amino acids. Northern blot analysis shows that the 11 KB PN1 transcript is abundant in DRG. Amplification of the PN1 sequence by RT-PCR indicates that PN1 is expressed in the CNS, as well as in sympathetic ganglia of the peripheral nervous system. Specifically, it is detected in brain, spinal cord, DRG, nodose ganglia, superior cervical ganglia, sciatic nerve, and heart, but not in skeletal muscle. The functional expression of PN1 in *Xenopus oocytes* and its cell distribution in DRG by *in situ* hybridization studies will be discussed.

POTASSIUM CHANNEL PHYSIOLOGY, PHARMACOLOGY AND MODULATION II

718.1

ACETYLCHOLINE ACTIVATES APAMIN-SENSITIVE K^+ CHANNELS IN CHICK COCHLEAR HAIR CELLS. W.A. Yuhas* and P.A. Fuchs, Dept. Physiol., U. Colorado Sch. Med., Denver, CO 80262.

Acetylcholine (ACh) hyperpolarizes cochlear hair cells by activating calcium-dependent potassium channels. It has been suggested that this mechanism employs the large conductance calcium-activated K^+ channels (maxi-K^+s) that support electrical tuning in hair cells of some nonmammalian vertebrates. We show here that this suggestion is unlikely to be correct since the K^+ current elicited by ACh ($I_{K(ACh)}$) differs both physiologically and pharmacologically from that flowing through maxi-K^+ channels ($I_{K(Ca)}$) in the same hair cells. The I-V relation for $I_{K(ACh)}$ is approximately linear through its reversal potential near E_K and has an "N-shape" that peaks near 0 mV. In contrast, the I-V relation for $I_{K(Ca)}$, flowing through maxi-K^+ channels, is sharply outwardly rectifying about the reversal potential at E_K and reaches its maximal value near +30 mV, suggesting different permeation and activation mechanisms. Substitution of internal K^+ with Cs^+ ions prevents $I_{K(Ca)}$ but not $I_{K(ACh)}$. The scorpion venom peptide charybdotoxin blocks half of $I_{K(Ca)}$ at 3 nM but has no effect on $I_{K(ACh)}$ at 65 nM. In contrast, the bee venom peptide apamin blocks half of $I_{K(ACh)}$ at 10 nM but leaves $I_{K(Ca)}$ unaffected at 1 μM. These data show that ACh activates K^+ channels that are distinct from maxi-K^+ channels, and are likely to be members of the small conductance class of calcium-activated K^+ channels. Supported by NIDCD DC 01508 and the National Organization for Hearing Research.

718.2

DEVELOPMENT OF Kv3.1-LIKE CURRENTS IN ACOUSTICO-VESTIBULAR NEURONS OF THE CHICKEN EMBRYO BRAIN IN VITRO R.Hendriks, W.Amin Hossain, D.K.Morest, L.K. Kaczmarek, R.M. Davidson and E-M. Ostapoff* Dept. Anat., Univ. of Connecticut Health Ctr., Farmington, CT 06030 & Dept. Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06520.

The *Shaw*-like, mammalian Kv3.1 channel is a voltage-dependent potassium channel, highly expressed in mature auditory brainstem nuclei. Associated currents have a high activation threshold, are slow to inactivate and are very sensitive to 4-AP and TEA. We have characterized these currents in the acoustico-vestibular anlage, containing the presumptive nuc. magnocellularis (NM, putative homologue to the mammalian anteroventral cochlear nucleus) at several developmental stages. Whole-cell patch recordings were made from normotypic cell cultures that were highly enriched with NM neuroblasts. Since these neuroblasts normally start to migrate at E5.5, explants were made on E4 and E7 and cultured for 2 days (E4/2D and E7/2D, respectively). They were identifiable microscopically as large, phase-bright, spherical cell bodies with neuritic processes. Sodium-like current transients, characteristic of neurons, were absent at E4/2D but pronounced at E7/2D for these cells. Subsequent recordings were performed in defined external saline solutions containing 1μM TTX (for E7/2D only) and zero-added calcium, to block voltage-dependent sodium conductances and calcium and/or calcium-dependent conductances, respectively. At E4/2D and E7/2D, step depolarizations revealed macroscopic outward currents that were a composite of two components: a fast initial transient current and a slowly inactivating current. The initial transient current could be fully revealed by pre-conditioning pulses to -100mV (Vh=-70mV) and eliminated with pre-conditioning pulses to -40mV. This A-like current was smaller at E4/2D than at E7/2D. The second component had Kv3.1-like properties, based on criteria for this current, including an activation threshold of about -10 mV. *In summary*, Kv3.1-mediated currents appear to occur very early in neuronal development, well before synaptic activity in ascending auditory pathways. Supported by T32DC00025, NS29613.

718.3

MICE LACKING THE DELAYED RECTIFIER POTASSIUM CHANNEL MKV1.1. S.L. Smart*, A. Messing, S.Y. Chiu, P. Schwartzkroin, B. Tempel. Depts. of Pharmacology, Otolaryngology, and Neurological Surgery, U. of Washington, Seattle, WA 98195; Depts. of Neurophysiology and Vet. Medicine, U. of Wisconsin, Madison, WI 53706.

The mouse delayed rectifier potassium channel mKv1.1 is widely expressed in the CNS and elsewhere, and is thought to be important in normal neuronal functions such as axonal conduction. In order to assess the consequences of the absence of this channel, the mKv1.1 gene open reading frame (ORF) was removed through targeted homologous recombination. A targeting construct was built containing 129Sv genomic DNA sequences flanking the mKv1.1 gene ORF, a neomycin resistance cassette, and a thymidine kinase gene. The construct was electroporated into cultured embryonic stem cells, which were then subjected to both positive and negative selection. Three of the surviving ES cell clones which screened positively by Southern blot for having undergone homologous recombination were injected into blastocysts for production of chimaeras. Chimaeric mice from two clones proved capable of germline transmission. The chimaeric founders have been mated with Black Swiss, 129Sv and C57/B6 mice, and homozygotes have been obtained from heterozygote intercrosses. Use of an mKv1.1 ORF probe on a Southern blot has confirmed the absence of the mKv1.1 gene ORF in homozygous animals. The homozygotes show increased mortality and a phenotype with possible motor and/or neurological components. Studies are underway to elucidate the physiological basis of the apparent phenotype.

718.4

TWO DISTINCT, OVERLAPPING "DELAYED-RECTIFIERS" DETERMINE THE VOLTAGE-DEPENDENT POTASSIUM CURRENT PHENOTYPES IN ST. LACUNOSUM-MOLECULARE INTERNEURONS IN PRIMARY CULTURE. A. Chikwendu* and C.J. McBain. NICHD-LCMN , NIH, Bethesda, MD 20892.

Whole cell, voltage-clamp recordings were made from primary cultures of dissociated st. L-M interneurons from rats P5-P10. In cells with a bipolar morphology, "delayed rectifier" outward K^+ currents were activated at test potentials positive to -40mV (V_h = -60mV). One of two current phenotypes was usually observed which we termed "sustained" and "slowly-inactivating". The voltage-dependence of activation of either current phenotype, however, was fit by the sum of at least two boltzmann equations suggesting that the total outward current resulted from the temporal overlap of multiple components. The total outward current showed minimal (~20%) voltage-dependent inactivation (Vhalf = -65 ± 3.8mV, n=14). In cells possessing predominantly "slowly-inactivating" outward currents, 4-AP dose-dependently (10μM - 30mM) blocked a fraction of the total outward current with an IC_{50} of 312 ± 101μM. At a maximal concentration of 30mM, 4-AP selectively blocked 41% of the total current. Isolation of the 4-AP sensitive component yielded a "slowly-inactivating" current with a positive voltage dependence of activation (Vhalf = 11.9 ± 1.4mV, n=4). The current remaining in 30mM 4-AP was "sustained" and also possessed a positive Vhalf of 6.2 ± 2mV (n=4). In cells where a "sustained" outward current dominated, 4-AP (30 mM) again removed a "slowly-inactivating" component which represented only 23% (n = 5) of the total outward current. TEA dose-dependently (10μM - 30mM) blocked the total outward currents with an IC_{50} of 142 ± 47μM. In 30mM TEA, 85% of the total outward current was blocked regardless of whether the current phenotype was predominantly "sustained" or "slowly inactivating". However, low concentrations of TEA (10 - 100μM) selectively removed the "slowly-inactivating" current component (Vhalf = 10.1 ± 1.3mV, n=5).

In conclusion, bipolar st. L-M interneurons have at least two "delayed rectifier" currents with similar activation profiles which can be differentiated based on their sensitivity to 4-AP and TEA. The differing proportions of either current component usually determines the overall current phenotype in any given cell.

718.5

DO CHANNELS FORMED BY Kv1.4 CONTRIBUTE TO THE TRANSIENT CURRENT IN ST. PYRAMIDALE INTERNEURONS ? L. Zhang* and C.J. McBain NICHD-LCMN, NIH, Bethesda, MD 20892-4495.

Homomeric channels formed by the voltage-dependent K^+ channel subunit Kv1.4 exhibit a transient current which is inhibited by decreasing external K^+ concentrations ($[K^+]_o$). Immunohistochemical studies have shown the Kv1.4 protein to be localized to cells tentatively identified as interneurons of the CA1 hippocampus. Here we have examined the effects of $[K^+]_o$ on the transient current of cells from subfield CA1 and correlated these effects with cell morphology in slices from neonatal rats (11-15 days). With whole-cell and outside-out patch voltage clamp techniques, transient currents were elicited by depolarization to potentials positive to -60mV (Vhold -90mV) and were isolated from sustained currents by use of different prepulse paradigms. Isolated whole-cell transient K^+ currents in various $[K^+]_o$ were compared with control transient current recorded in 3.5 mM $[K^+]_o$. 38% of all the cells recorded (n = 78) and whose morphology was subsequently identified by biocytin labeling showed a selective reduction (36%) or increase (62%) in the amplitude of the transient current measured at 0mV when perfused with a K^+-free or a 10 mM $[K^+]_o$ solution, respectively. Analysis of the voltage-dependence of this transient current revealed a Vhalf of -20 ± 2.1 mV (n = 11). Of these cells, 60% were identified as inhibitory interneurons located close to the border of st. pyramidale, 17% were observed in st. radiatum proper, and 10% were CA1 pyramidal neurons. In all other cells the transient current was increased in K^+-free conditions as would be expected. Current-clamp recordings from cells exhibiting a decrease in transient current in K^+-free solution in voltage-clamp studies (n = 3) demonstrated an increase in their action potential duration (18%) concomitant with an augmentation of the afterhyperpolarization amplitude (52%) in K^+-free solution. In addition K^+-free solution also caused a membrane hyperpolarization of ~3 mV. Our data suggest that external K^+ can modulate a transient K^+ current presumably arising from Kv1.4 subunit expression in hippocampal CA1 neurons. This current contributes to the total transient current and participates in the action potential repolarization in inhibitory neurons of st. pyramidale.

718.6

PERMEATION PROPERTIES OF THE M-CURRENT IN RAT SUPERIOR CERVICAL GANGLION NEURONS.

R. Cloues*, J.P. Adelman, and N.V. Marrion. Vollum Institute, OHSU, Portland, OR 97201.

The M-current is a voltage-dependent potassium (K^+) current which is inhibited by muscarinic acetylcholine receptor activation. We have examined aspects of M-channel permeation including the relative permeabilities of various monovalent cations and whether the M-channel displays anomalous mole fraction behavior. Whole-cell M-currents were recorded from cultured rat superior cervical ganglion (SCG) neurons using the nystatin perforated-patch technique. To determine relative permeabilities, cells were bathed in a solution containing 135 mM NMG (a non-permeant cation) and 15 mM of the cation of interest (acetate salt), as well as 5 mM MgAc, 0.1 mM CaAc, 0.2 mM CdAc, and 10 mM 4-AP (to block other voltage-dependent K^+ channels). Shifts in M-current reversal potential were measured and the permeability of different cations relative to K^+ determined. The permeation sequence of the M-current is: $Tl^+ > K^+ > Rb^+ > NH_4^+ >> Na^+$. For example, Rb^+ is less permeable than K^+ ($P_{Rb}/P_K = .78$), blocks conduction through the channel ($g_{Rb}/g_K = .40$), and slows the deactivation tail currents at -50 mV from 107 ± 15 ms to 136 ± 18 ms (sem; n=4; $P < 0.1$). The M-current displays anomalous mole fraction behavior, a property suggestive of a multi-ion pore. In these experiments, M-currents were recorded in isotonic K^+ (150 mM) and then titrated with increasing amounts of Rb^+. M-current conductance goes through a minimum as the mole fraction of Rb^+ varies from 0 to 1.0. The M-channel thus displays many of the properties of other voltage-dependent K^+ channels.

718.7

CHARACTERIZATION OF THREE DISTINCT K^+ CURRENTS MODULATED BY ATP IN HUMAN NEOCORTICAL NEURONS.

C. Jiang* & G. G. Haddad, Department of Pediatrics, Section of Respiratory Medicine, Yale University, New Haven, CT 06510

ATP-modulated K^+ channels play an important role in regulating membrane excitability and neuronal responses to metabolic stress. In order to study the presence and modulation of these K^+ channels in *human* neocortical neurons, single channel K^+ currents were recorded in the excised patch configuration from dissociated human neocortical neurons. Three outward currents sensitive to ATP were characterized. All channels were selectively permeable to K^+. One of them had an unitary conductance of ~45pS and showed a strong inward rectification with symmetric K^+ across the membrane. This K^+ current was inhibited by ATP with IC_{50} of ~150μM, and channel activity was suppressed by non-hydrolyzable ATP analogue AMP-PNP and glibenclamide. The second K^+ current had a conductance of ~170pS, but no rectification was seen. This current was also inhibited by ATP, AMP-PNP and glibenclamide. Unlike the 45pS current, however, activation of this current required the presence of μM Ca^{++} on the cytosolic side. The third K^+ current was Ca^{++} dependent, had a conductance of ~260pS and was inhibited by charybdotoxin. Unlike the other 2 channels, ATP (0.03-1.0mM) *enhanced* channel open probability and increased the unitary conductance. These results indicate that a number of K^+ channels are regulated by physiologic concentrations of ATP in human neocortical neurons. (Supported by NIH P20 NS32578, HD15736)

718.8

HIERARCHY OF GLUTAMATE AND NORADRENERGIC RECEPTOR FUNCTION IN HIPPOCAMPAL CA1 NEURONS. R. Nouranifar*, T. Wong, R.D. Blitzer and E.M. Landau. Dept. of Psychiatry, Mt. Sinai School of Medicine and Bronx VAMC, New York NY 10029

Intracellular recording techniques were used to investigate the interaction between the metabotropic glutamate and β-adrenergic signaling pathways in pyramidal cells from the CA1 region of adult rat hippocampal slices. Both trans-1-aminocyclopentane-1S,3R-dicarboxylic acid (ACPD) and isoproterenol (ISO) blocked the slow afterhyperpolarization (sAHP) which followed membrane depolarization when applied individually. However, their effects were less than additive when applied in combination. The effect of the combined agonists became additive when protein kinase C (PKC) was inhibited by PKC_{19-31} in the recording electrode. The involvement of PKC confirmed by the bath application of chelerythrine, a specific inhibitor of PKC, which also rendered the combined effect of ISO/ACPD additive. Interestingly, the inhibitory effect of ACPD on AHP was not blocked by chelerythrine, which did prevent the blockade of the AHP by phorbol-12,13-dibutyrate (PDBu). The ISO-ACPD interaction could be mimicked by the combination of ISO and PDBu, and most importantly, by 8-bromo-cAMP and PDBu. The results indicate that the metabotropic glutamate pathway coupled to PKC prohibits the β-adrenergic inhibition of the AHP.

The demonstration that PKC can prevent an effect of the PKA pathway reveals a potentially important motif of interaction between these two pathways, expressed as an assignment of rank to different neurotransmitters. Thus, a neurotransmitter of a higher rank (glutamate, coupled to PKC) can override the effect of a lower-ranking transmitter (norepinephrine, coupled to cAMP), enabling the neuron to discriminate among neurotransmitters released in temporal proximity. Such interacting effects on the AHP might function as a protective mechanism, preventing over-stimulation of pyramidal neurons upon release of multiple excitatory neurotransmitters. Supported by NARSAD and a VA Merit Grant.

718.9

Properties of Ca^{2+}-dependent, large conductance K (BK) channels in cultured rat melanotrophs. S.J. Kehl*, K. Kheirani, M. Mitton, and K. Wong, Department of Physiology, UBC, Vancouver, B.C., Canada, V6T 1Z3

The biophysical and pharmacological properties of BK channels were studied by using the patch clamp technique. In excised patches the open probability (P_o) was increased by depolarization and/or by increasing $[Ca^{2+}]_i$. The current/voltage relationship in symmetrical K^+ (150 mM) was linear between -60 and 60 mV and had a slope of 267 pS (95% C.L. 258-275 pS). Relative permeabilities, estimated from the reversal potential obtained either directly or by extrapolation in biionic conditions in which the test cation completely replaced internal K^+, gave a permeability sequence of K^+ (1) > Rb^+ (.87) > NH_4^+ (.17) > Cs^+ > Na^+ (<.02). Outward Rb^+ currents had a slope conductance of 30-40 pS suggesting a long mean dwell time for Rb in the pore. Outward currents were not seen with either Cs^+ or Na^+. External TEA^+ caused a weakly voltage-dependent open channel block. The K_D for the block at 0 mV was 260 μM and the slope of the Hill plot was 0.82 implying that the block was produced by the binding of a single molecule of TEA^+. Internal TEA^+ (80 mM) had no effect. External charybdotoxin (10-40 μM) caused a slow block of BK channels. Difficulty in obtaining a stationary P_o with outside-out patches precluded a systematic study of the binding kinetics of charybdotoxin.

718.10

CALCIUM-ACTIVATED POTASSIUM CHANNELS IN FERRET RETINAL GANGLION CELLS. G-Y Wang, D.W. Robinson, J.M. Horowitz* and L.M. Chalupa. Neurobiology, Physiology and Behavior and Center for Neuroscience, University of California, Davis, CA95616.

Patch-clamp techniques were used to study the calcium-activated potassium currents in retinal ganglion cells (RGCs) retrogradely labeled by prior injection of rhodamine latex beads. In the cell-attached configuration, with 140mM K^+ in the electrode and 5.9mM K^+ in the bathing solution, two types of calcium-activated potassium channel were identified. These channels had conductances of 118pS (BK) and 22pS (SK) and were sensitive to increases in intracellular calcium concentration resulting from the addition of Bay-K8644 to the bathing solution. In the inside-out configuration the open probability of both channels increased as intracellular patch calcium concentration was increased. In the current-clamp mode, bath application of charybdotoxin, a selective blocker of BK channels, significantly increased the firing frequency of RGCs which responded in sustained manner to maintained depolarization. In contrast, charybdotoxin had little or no effect on the discharge patterns of RGCs which responded to such current injections in a transient fashion. These results demonstrate that the BK channel of the calcium-activated potassium conductance regulates the firing frequency of sustained RGCs. Supported by NIH grant EY-03991 to LMC

718.11

ATP-SENSITIVE K^+ CHANNELS OF HUMAN SKELETAL MUSCLE FIBERS: AGE-DEPENDENT CHANGES OF CHANNEL PROPERTIES. D. Tricarico*, R. Petruzzi and D. Conte Camerino. Unit of Pharmacology, Dept. of Pharmacobiology, Univ of BARI, via Orabona n° 4, I-70125 BARI, ITALY.

ATP sensitive K^+ channels (K_{ATP}) are the most abundant K^+ channels in skeletal muscle were may differently respond to various metabolic insults. An abnormal functionality of K_{ATP} channels has been proposed to contribute to the pathogenesis of hypokalemic periodic paralysis (HOPP), in which the primary cause involve a mutation of DHP receptor. However, at present, the link between the channel mutation, that results in an increase of inactivation of Ca^{2+} channels (Sipos et al., *Biophysical J.* 68/2: A209, 1995), the muscle paralysis and the lowering of the serum K^+ concentration is still obscure. In the present work, a preliminary screening of human K_{ATP} channels of skeletal muscle fibers has been performed by patch clamp technique. The experiments were carried out on biopsies of human skeletal muscle removed, under general anaesthesia, from vastus lateralis of patients who required a orthopedic surgery. Single channel recording were performed in inside-out configuration at constant voltage (-60 mV), at 20 °C, in symmetrical KCl 150 mM or during voltage step going from -70 mV to + 70 mV (Vm). The biopsy from a 17 year old man, showed an high K_{ATP} channel activity (mean patch current, $I_{\cdot}$: 37 pA) due to a 64 pS channel. The channel density ($N/_{area}$) was 12 and the open probability (P_{open}) was 0.313. The IC_{50} for ATP was about 50 μM. The patient did not show abnormality in the level of the serum metabolites nor in the concentration of K^+ ion being 5.4 meq/L. In contrast, the biopsy from a 67 year old woman showed a lower K_{ATP} channel activity (I : 14,4 pA) due to a decrease of $N/_{3\,\mu m^2}$ that was 3.5, and P_{open} that was 0.0312. The single channel conductance did not change with aging being 61 pS. Moreover, the sensitivity of the channel for ATP was about 50 times higher. The patient did not show abnormality in the levels of the serum metabolites while the plasma concentration of K^+ ion was lower being 4.7 meq/L. Our data suggest that, as observed in aged rat skeletal muscle in which a decrease of $N/_{3\,\mu m^2}$ and P_{open} was found (D. Tricarico and D. Conte Camerino, *Mol. Pharmacol.* 46:754, 1994), the aging process alter also the K_{ATP} channels of human skeletal muscle. We also observed that the decrease of K_{ATP} channel activity found in aged human skeletal muscle is paralleled to a decrease of plasma K^+ level. The financial support of Telethon-Italy (Grant no. 579) is gratefully acknowledged.

718.12

DEPRESSION OF POTASSIUM CURRENT BY 5-HT7 RECEPTOR STIMULATION IN CULTURED RAT SUPRACHIASMATIC NEURONS. Y. Edagawa*, H. Saito and H. Katsuki. Dept. of Chem. Pharmacol., Fac. of Pharmaceut. Sci., The Univ. of Tokyo, Bunkyo-ku, Tokyo113, Japan

The suprachiasmatic nucleus (SCN) receives a serotonergic projection from the mesencephalic raphe nuclei and this input is thought to be important in circadian rhythms regulation. We performed the whole-cell recordings from the cultured postnatal rat SCN neurons to investigate the possible modulation of delayed rectifier potassium current (IK) by serotonin (5-HT). Sustained outward current induced by depolarization to 0mV by pulse stimulus for 500ms was identified as IK, since it was greatly inhibited by tetraethylammonium treatment. Administration of 5-HT depressed IK in a dose-dependent manner (10^{-7} to 10^{-5}M). 8-OH-DPAT (5-HT1A,7 agonist, 10^{-7} to 10^{-5}M) and 5-CT (5-HT1A,1B,7 agonist, 10^{-7} to 10^{-5}M) had similar effects of depression by 5-HT, but DOI (5-HT2 agonist, 10^{-5}M) had no significant effect. Ritanserin (5-HT2,7 antagonist,10^{-6}M) inhibited the suppressive effect by 8-OH-DPAT (10^{-6}M), whereas pindolol (5-HT1A,1B antagonist, 10^{-5}M) did not. The effect of 5-HT (10^{-6}M) was also inhibited by ritanserin (10^{-6}M), but not by pindolol (10^{-5}M) or MDL72222 (5-HT3,4 antagonist, 10^{-5}M). Forskolin, adenylyl cyclase stimulator, and 8-Br-cAMP, membrane-permeable cAMP analog, also reduced the IK.The effect of 5-HT and forskolin were not additive. Moreover, the inhibitory effect by 5-HT was attenuated by H-8, potent inhibitor of protein kinase A. These results indicate that the depression of IK is induced by the stimulation of 5-HT7 receptors, which is linked to intracellular cAMP-coupled mechanisms.

718.13

DIFFERENTIAL DEPLOYMENT OF FAST POTASSIUM CHANNELS ON CELL BODIES AND AXONS OF ADULT RAT DORSAL ROOT GANGLION NEURONS. B. Everill, D. Donnelly*[1] and J. D. Kocsis. Depts. of Neurology & Pediatrics[1], Yale Univ. Med. Sch., New Haven, CT. 06510, and Neuroscience Res. Ctr., VAMC, West Haven, CT. 06516.

Myelinated rat peripheral nerve fibers have a variety of kinetically fast and slow potassium channels (Roper, J. and Schwartz, J.R. 1989), but little is known about the K^+ channel organization of the cell bodies of these axons. Recently, several workers have indicated that Na^+ channel and GABA receptor organization of dorsal root ganglia (DRG) neurons change in response to axotomy. The present study was undertaken to characterize K^+ currents on the cell bodies of DRG neurons as a precursive examination of injury-induced plasticity of these currents. DRG neurons were dissociated from adult female Wistar rats and prepared for short-term (10-24 hrs.) examination. Whole cell patch clamp recordings were obtained from medium-sized neurons which give rise to myelinated peripheral axons. K^+ currents were isolated by blocking Na^+ and Ca^{2+} currents with appropriate ion replacement and channel blockers. The neurons were held at -80 mV and depolarized in 10 mV step (100 ms duration) increments to +20 mV. A prominent TEA-sensitive noninactivating delayed outward current was observed, and an outward leak conductance remained after application of 40 mM TEA inside and outside of the neurons. Little evidence for a fast 4-aminopyridine-sensitive K^+ current was observed using the voltage clamp protocol or those designed specifically to identify A-currents. Intra-axonal recordings from myelinated sensory axons in the root revealed a prominent 4-AP sensitive after-hyperpolarization suggesting the presence of fast K^+ currents on these axons. The presence of prominent transient 4-AP-sensitive currents on axons of these neurons and their absence or paucity on the cell body suggests that adult DRG neurons primarily deploy fast K^+ currents on their axons and not their cell bodies. Supported in part by the VA and the NIH.

718.14

CORTICOSTERONE EFFECTS LEAK BUT NOT Q CURRENT IN RAT HIPPOCAMPAL CA1 NEURONS. S.Purnyn, S. Beck.* Dept. of Pharmacology, Loyola University Medical School, Maywood, IL 60153.

Basal leak (I_{leak}) and Q currents (I_Q) were studied in CA1 neurons from rat hippocampal slices using patch-clamp technique in whole cell configuration. These currents were compared in cells from rats that were naive, sham operated (Sham), adrenalectomized (ADX) or ADX with corticosterone (CT) replacement by subcutaneous pellet implantation (ADX+CT).

I_Q was defined as a Cs^+ (EC_{50} = 0.33 mM) sensitive inward current induced by hyperpolarizing voltage steps from a holding potential of -65 mV. Delay in activation, but not in inactivation of this current was apparent. I_Q activation at -100 mV was best fit to second order kinetics. The time constant of I_Q activation was highly temperature dependent (Q_{10}=5) and it lost it's voltage dependence at 32°C. Cs^+ inside the cells was not able to block I_Q in concentrations sufficient to block the delayed rectifier. Substitution of intracellular K^+ with Na^+ did not change the amplitude of I_Q. No significant difference in the amplitude of I_Q was found between Sham, ADX and ADX+CT animals.

I_{leak} was isolated by subtracting I_Q from the current responses evoked by hyperpolarizing voltage steps used to study I_Q. In -65 to -110 mV voltage region I_{leak} V-I characteristic appeared to be linear with a reversal potential (Er) equal to the membrane resting potential of -63.2 mV and had a slope of 21.6 nS in cells from the Sham treated animals. I_{leak} showed temperature dependence with Q_{10} of 1.85 and no sensitivity to Cs^+ at 2mM concentration. Replacing intracellular K^+ with Cs^+ dramatically decreased I_{leak} conductivity. Replacing intracellular K^+ with Na^+ had no effect on conductivity but slowly shifted Er in a positive direction. I_{leak} conductivity was significantly lower in ADX+CT treated animals (14.8 nS). The reversal potential did not change. Decreasing I_{leak} might be responsible for increasing excitability of the cells by CT. Supported by USPHS NS28512 and KO2MH00880 to SGB.

718.15

AN INACTIVATING INWARD-RECTIFYING K CURRENT IN RAT LACTOTROPH CELLS IN PRIMARY CULTURE. J.R. Schwarz , B.J. Corrette, C.K. Bauer (SPON: European Neuroscience Association), Inst. Physiol., UKE, D-20246 Hamburg, Germany

Whole-cell patch-clamp experiments were performed in lactotroph cells from primary cultures of pituitaries from lactating rats. Cells were identified as lactotrophs by staining using a prolactin antibody (NIDDK, Baltimore, MD). Cells were kept in culture for up to 5 weeks. Throughout this period, an inward-rectifying K current (K_{IR}) was recorded in lactotrophs using isotonic KCl as external solution. This current differed from cell to cell with respect to current density and rate of inactivation. Inactivation starts at about -70mV and becomes faster and more complete with more negative potentials. This inactivation is independent of external Na^+. The fast-inactivating current type in lactotrophs strongly resembles the inward-rectifying K current of GH_3B_6 rat somato-mammotrophic tumor cells (Bauer et al., J. Physiol. 429:169, 1990). Changing the external K^+ concentration from 150 to 15 mM (K^+ exchanged by Na^+) produced a mean shift in the reversal potential of -55.2 mV, demonstrating a high selectivity of the recorded current for K^+ over Na^+. K_{IR} is effectively blocked by 5 mM Ba^{++}, but 0.5 mM Ba^{++} reduced the peak current by only 10-20%. 10 mM TEA partially (<50%) blocks K_{IR}.

A physiological role of K_{IR} in the control of prolactin secretion is suggested by the fact that the secretagogue TRH inhibited K_{IR} in about half of the lactotrophs tested. This inhibition might underlie membrane depolarization resulting in a TRH-induced enhanced Ca^{++} influx into the cell, as has been observed in a proportion of lactotrophs (Reid et al., in prep.).

718.16

Ca^{2+}-ACTIVATED POTASSIUM CHANNELS UNDERLYING AFTERHYPERPOLARIZATION IN NEOCORTICAL PYRAMIDAL NEURONS. Jian Kang*, John Huguenard and David A Prince, Department of Neurology and neurological sciences, Stanford University, Stanford, CA 95304

To investigate the ion channels underlying afterhyperpolarizations (AHPs) we utilized dual patch clamp recordings with two electrodes , a whole-cell current clamp and a cell-attached patch, on single rat neocortical neurons in visualized slices. The large conductance Ca^{2+}-activated K^+ channel (BK) has been hypothesized to contribute to repolarization and fast afterhyperpolarization in neurons because it opens after a single spike in GH_3 cells and dissociated hippocampal neurons (Lang & Ritchie, Pflüger Arch 1987; Yoshida, Jap. J. Physiol. 1991). We found that the BK channel was not triggered by depolarization induced spikes in pyramidal neurons in slices. Instead, an intermediate conductance Ca^{2+}-activated K^+ channel (IK) with a conductance of 64 pS was seen immediately following the AP transient and with mean post-spike channel activity lasting 9.0±0.9 ms. A small conductance Ca^{2+}-activated K^+ channel with a conductance of 29 pS (SK) was also observed immediately following the AP transient or IK openings, and the activity of SK lasted 94.5±6.3 ms. The same results were also obtained by synaptically evoked spikes and at different temperatures. Spike-related openings of IK and SK were markedly reduced by superfusion of 0.3 mM Cd^{2+}. In contrast, the BK channel only opened as neuronal responses deteriorated eg. smaller or absent spikes and in a spike-independent manner. The membrane potential became hyperpolarized (>-10 mV) during BK channel activity. Inside-out patches excised from slices or dissociated neocortical pyramidal neurons also contained three types of Ca^{2+}-activated K^+ channels. IK and SK are more sensitive to Ca^{2+} than BK. Similar to BK, IK was also voltage-dependent and blocked by 1 mM TEA or 100 nM Charybdotoxin (ChTX) in the pipette solution. SK was voltage-independent and insensitive to TEA, ChTX or apamin. We conclude that IK and SK respond to the transient intracellular Ca^{2+} elevation elicited by the action potential and contribute to the two components of AHPs. BK does not contribute to the AHP during short periods of stimulation.

718.17

TWO CLASSES OF BK CHANNELS IN RAT NEURO-EPITHELIAL CELLS. J.-M. Mienville* and J.L. Barker. Lab of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

In situ patch-clamp recordings from E12-14 rat telencephalon reveal two classes of large-conductance Ca-activated K (BK) channels: the first one represents only a very small percent (~1%) of occurrences and displays properties of classical BK channels, i.e., normal gating mode, sensitivity to charybdotoxin (CTX), absolute requirement of Ca for activation, and voltage dependence of 10-15 mV per e-fold increase in Po. In the second class, channels exhibit a *buzz* gating mode and, as described previously (J. Physiol. **481**: 293-298), switch to normal mode upon exposure to intracellular trypsin. These channels appear to be insensitive to CTX, are capable of activation in the absence of cytoplasmic Ca, and display voltage dependences >40 mV per e-fold increase in Po. An interesting feature is that their Po can be "titrated" with intracellular trypsin, longer enzyme applications yielding lower Po's. Following treatment, these channels are stimulated by increases in cytoplasmic Ca. Because of its overwhelming presence in embryonic tissue, this channel subclass may encompass immature forms of BK channels.

718.18

THE HYPERPOLARIZED SHIFT OF I_A INACTIVATION IN HYPERTENSIVE RATS IS NOT SECONDARY TO ELEVATED BLOOD PRESSURE. Walter P. Robertson and Geoffrey G. Schofield*. Department of Physiology, Tulane University School of Medicine, New Orleans, LA 70112.

Increased sympathetic nerve activity has been implicated in the development and maintenance of hypertension. Consistent with this hypothesis, I_A in superior cervical ganglion neurons from adult spontaneously hypertensive rats (SHR) inactivates at more hyperpolarized potentials than in normotensive WKY rats. In neurons from young, pre-hypertensive SHR rats the mean I_A half inactivation potential (V_h) was not different than that recorded from neurons of young WKY (-76.4 ± 2.4 and -78.5 ± 2.1, respectively). These data suggest that alterations in I_A gating are not causative of hypertension in the SHR. However, new preliminary data shows that the V_h for I_A inactivation does shift in adult SHR rats prevented from developing hypertension by prolonged treatment with the anti-hypertensive drug enalapril. Thus the hyperpolarizing shift of I_A inactivation is not a secondary adaptation to the elevated blood pressure.

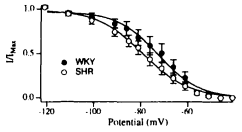


Figure. H infinity plots of peak A-current. I_A was recovered for 1.0 sec prior to a step to -30 mV. Rats received 25 mg/L enalapril in drinking water from 3-4 weeks of age until use. Mean V_h for SHR neurons was -79 ±3 mV and -72 ± 4 mV for WKY neurons. Solid lines are a least-squares fit of the data to a Boltzmann function.

718.19

POTASSIUM CONDUCTANCES PLAY IMPORTANT ROLES IN PRODUCING MAGNETIC FIELDS IN THE GUINEA PIG HIPPOCAMPAL LONGITUDINAL CA3 SLICE. J. Wu* and Y. C. Okada. Depts. Neurol. & Physiol., Univ. New Mexico Sch. Med., Albuquerque, NM 87131

We analyzed the influence of K^+-conductances on size and shape of the magnetic evoked fields (MEFs) in the guinea pig longitudinal CA3 slice. Evoked potentials (EPs) were recorded simultaneously with the MEFs to compare their similarities in timing and temporal waveforms. Intracellular recordings were carried out to gain further insight into the cellular currents generating the MEFs. The stratum lucidum of rectangular CA3 slice was stimulated by single pulses (1.0 mA, 50 μs, 0.1 - 0.5 Hz) with six pairs of bipolar electrodes in Ringer solution (concentrations in mM: NaCl 124, KCl 5, $NaHCO_3$ 26, NaH_2PO_4 1.2, $MgCl_2$ 0.6, $CaCl_2$ 2.5, glucose 10, 36-37°C) containing 0.1 mM picrotoxin. The MEF consisted of an initial component with 5 ms peak latency followed by a biphasic slower component lasting 100 ms. 0.1 mM 4-aminopyridine (4-AP), which selectively blocks $g_{K(A)}$, reversibly enhanced the slower component of both the MEF and EP. 1 mM tetraethylammonium (TEA), which blocks $g_{K(C)}$, reduced the initial MEF component, but increased the amplitude of the slower component of both the MEF and EP. Blocking of $g_{K(AHP)}$ with 1 μM cabachol was demonstrated in our preparation with intracellular recording. The same concentration prolonged the latency of the slower biphasic MEF component with some increase in amplitude. The latency of EP response was also prolonged and its amplitude was clearly enhanced. These results indicate that: (1) the blockade of $g_{K(A)}$ increases the duration of depolarization after the opening of g_{Na}, thereby increasing the probability of opening g_{Ca} and consequently increasing the MEF amplitude; (2) the blocking of $g_{K(C)}$ may lead to an increase in the duration of the Ca^{2+}-activated action currents, enhancing the magnitude of the slower MEF component; (3) the blockade of the long-lasting afterhyperpolarization may prolong the voltage-dependent Ca^{2+} channel open time, thereby broadening the slower component of MEFs. We conclude that active conductances are important in interpreting MEFs.
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718.20

HODGIN-HUXLEY REDUX: EXCITABILITY OF THE SQUID GIANT AXON REVISITED. J.R. Clay*. NINDS, NIH, Bethesda, MD 20892 and MBL, Woods Hole, MA 02543.

The Hodgkin and Huxley model (J. Physiol. 117:500, 1952) is generally believed to provide a good description of the excitability properties of the squid giant axon. This view is incorrect. Specifically, the model predicts a steady train of action potentials (AP's) in response to a sustained current pulse of sufficient amplitude, whereas the axon itself generates only a single AP for these conditions regardless of pulse amplitude or duration. The underlying mechanism for this result concerns, primarily, I_K, which requires significant revision in the model. Specifically, the I_K current-voltage relation is a non-linear function of $(V-E_K)$, which is well described by the GHK equation (Clay, J. Physiol. 444:499, 1991). This result, in turn, gives a much steeper activation curve for I_K than in the HH model, in particular a steep rising phase at -45 mV. Consequently, I_K acts, effectively, as an impedance shunt during a sustained current pulse as the membrane potential is depolarized from the foot of the initial spike. I_{Na} is thereby shortcircuited, and the membrane quiescently rests in the -55 to -45 mV range, depending upon pulse amplitude. The squid giant axon is, therefore, a good model system for rapid accommodation, rather than bursting, or tonic firing behavior.

POTASSIUM CHANNEL PHYSIOLOGY, PHARMACOLOGY AND MODULATION III

719.1

ARE K^+ CHANNEL β-SUBUNITS NAD(P)H-DEPENDENT OXIDOREDUCTASE PROTEINS ? T. McCormack[1], K. McCormack[2]*, H. Moreno[1,3], and B. Rudy[1]. 1) Dept of Physiology and Neurosciences and Dept. of Biochemistry, New York University Medical Center, NY NY 10016 2) Dept. Genetics, University of Wisconsin, Madison , WI. 3) Dept Fisiologia Universidad Nacional de Colombia-CIF.

We have observed that Shaker K channel β-subunits (β1, β2 and β3) show homology to members of an NAD(P)H -dependent oxidoreductase superfamily. We have also identified EST sequences from rice and Arabidopsis to which β-subunits show 40-55% identity over the 96 amino acids encoded by these partial cDNA's. We are interested in determining whether K^+ channel β-subunits are functional enzymes and whether pyridine-nucleotide cofactors play a role in modulating K^+ channels through β-subunits. In order to pursue these studies we have cloned human homologs of the rat β-subunits. The β1 gene is complex and generates alternatively spliced products with different amino termini and tissue-specific expression. The β1 product is expressed mainly in brain, while β3 is most abundant in heart. Additional studies will focus on the physiological roles of β-subunits and on the interactions of specific α- and β- subunits.
Supported by a Grant in Aid from the American Heart Association and NIH grant NS 30989.

719.2

THE MODULATION OF HUMAN (*hslo*) CA^{2+}-ACTIVATED K^+ CHANNELS BY PROTEIN KINASES AND ATP ANALOGS. T.J. DiChiara* & P.H. Reinhart, Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710 USA.

We have investigated the modulation of human Ca^{2+}-activated K^+ channels (the hbr5 splice variant of hslo) in a stably transfected HEK293 (human embryonic kidney) cell line. Using inside-out patch clamp analysis we have shown that these channels can be up-modulated to a similar extent by either protein kinase A (PKA) or protein kinase C (PKC). Both of these kinases increase the steady-state Ca^{2+}-dependence of these channels, and also have profound effects on the channel activation kinetics (n=4). When added in the absence of protein kinases, ATP does not up-modulate hslo channels, however, it slows channel 'run-down' measured as a right-shift in macroscopic g/g_{max} curves (28 of 31 experiments). As soon as ATP is washed out, 'run-down' continues. ATPγS has a similar, although less robust, effect which is resistant to wash-out (n=12). Three other non-hydrolyzable ATP analogs AMP-PNP (n=11), AMP-PCP (n=3), and AMP-CPP (n=3) have no effect on the rate of run-down nor on peak currents. These data indicate that human Ca^{2+}-activated K^+ channels can be targeted by numerous signal transduction cascades. Furthermore the data show that ATP itself also modulates hslo channels, and that this effect is distinct from modulation by PKA and PKC. The mechanism of ATP modulation appears to involve protein phosphorylation rather than a direct effect of nucleotide binding. Supported by NIH grant NS31253 to PHR.

719.3

REGULATION OF rKv 1.6 AND rKv 3.1 EXPRESSED IN *XENOPUS* OOCYTES BY PROTEIN KINASE C. A.C.S. Costa*, L.C. Starke and P.J. Pfaffinger, Baylor College of Medicine, Houston, TX 77030.

The regulatory actions of PKC on cloned rKv 1.6 and rKv 3.1 were studied here with both electrophysiological and biochemical approaches. By using conventional two electrodes voltage-clamp, we showed a 40-50% reduction in peak amplitude of currents recorded from oocytes expressing either rat K^+ channel clones after 15-20 minutes incubation in external solution containing phorbol-12-myristate, 13-acetate ester (50 nM). In contrast, rKv2.1 was not suppressed under these conditions. However, to avoid possible artifacts due to PKC stimulated membrane internalization, we switched to studying regulation in inside-out excised macropatchs. Patches were excised from oocytes expressing either rKv 1.6 or rKv 3.1 and then tested with internal solutions containing a partially purified preparation of PKC in an optimized low Ca^{2+} buffer. The kinase activity in this solution was 25% of the Ca^{2+} stimulatable activity. Where patches could be held long enough, the current could be reversed upon kinase removal. The reversible nature of the regulation, and recording in excised, low Ca^{2+} solutions, demonstrates that the effect is not due to internalization. To begin to characterize the PKC sites on the channels, we have performed *in vitro* phosphorylation assays of a purified N-terminus fragment of rKv1.6. This protein only shows a slight ability to be phosphorylated, around 1% phosphorylated. We are further refining this biochemical strategy, along with mutagenesis, to better characterize the mechanism of regulation by PKC.

Supported by NIH RO1-NS 31583, Baylor Mental Retardation Center P30-HD24064, Klingenstein Fellowship Award.

719.4

PHOSPHATASE 2B (CALCINEURIN) PROMOTES LOW OPEN PROBABILITY BEHAVIOUR IN LARGE CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM CHANNELS IN CULTURED HIPPOCAMPAL NEURONS. G.A.Hicks, B.A.Perrino, and N.V. Marrion*. Vollum Institute, O.H.S.U., Portland, Oregon 97201.

Phosphorylation and dephosphorylation can modulate the activity of large conductance calcium-activated potassium (BK) channels. We are investigating the potential role of the calcium/calmodulin dependent phosphatase, calcineurin, in modulation of BK channels in rat hippocampal neurons. BK channel activity was recorded from 'inside-out' patches in isotonic potassium with Ca^{2+} buffered with EGTA. Channel open probability (Po) was dependent upon membrane potential and the [Ca^{2+}] in the bathing solution. For example, in 3 patches held at +20mV, the mean Po was 0.25 in the presence of 1μM Ca^{2+} and 0.92 in 10μM Ca^{2+}. Channel activity was, however, observed to vary significantly between patches under the same conditions of membrane potential and [Ca^{2+}]. With a [Ca^{2+}] of 1μM, channel Po ranged from 0.02 to 0.66 at a membrane potential of +20mV. This variability suggests that channel activity may be under the control of factors other than voltage and [Ca^{2+}].

A calcium-calmodulin independent form of calcineurin (92 or 120nM) was applied to patches in the presence of either 1 or 10μM [Ca^{2+}] in which Po was >0.3. In 6 out of 8 patches a reduction in Po was observed. The reduction was either sustained (n=4) for the duration of the calcineurin application, or transient (n=2), channel activity returning to control levels before application was ceased. The vehicle alone had no effect (n=4). The sustained response consisted of a 50-100% reduction in Po and was fully reversed in 3 of the 4 patches when calcineurin application was ceased. The transient response consisted of one or more transient reductions (30-90%) in Po over the first minute of calcineurin application followed by a return to control levels. Preliminary evidence suggests that the reduction in Po is due to an increase in closed duration with no change in mean open time. Both the reversal of the sustained response and the transient nature of other responses suggests that an active kinase and ATP may be present in the excised patch. Inhibitors of protein kinases will be used to investigate this possibility. (This work was supported by The Wellcome Trust.)

719.5

PHOSPHORYLATION OF CB-1 BY PROTEIN KINASE C. K. Mackie*, D.E. Garcia, A. Perdichizzi, P. Hill+, and B.J. Murphy, Departments of Anesthesiology, Physiology and Biophysics, and Pharmacology, Univ. of Wash., Seattle, WA 98195-6540. +Panlabs, Bothell, Wa 98011-8805.

Neurotransmitters that stimulate protein kinase C (PKC) in neurons disrupt activation of inwardly-rectifying potassium currents (K_{ir} current). In AtT-20 cells transfected with rat brain cannabinoid receptor (CB-1), stimulation of PKC by pretreatment with 100 nM PMA blunted the activation of K_{ir} current: In cells treated with the inactive phorbol ester, 100 nM 4α-phorbol, the cannabimimetic, 100 nM WIN 55,212-2, increased K_{ir} current (5.2± 0.9 pA/pF, n=23) while in cells treated with the active analog, 100 nM PMA, WIN 55,212-2 stimulation was blunted (2.2±0.5 pA/pF, n=16). This may be a consequence of CB-1 phosphorylation by PKC. To address this issue we studied phosphorylation of CB-1-glutathione-S-transferase (GST) fusion proteins corresponding to the intracellular domains of CB-1. GST was not a substrate for PKC. The fusion protein corresponding to the third intracellular loop was heavily labeled by PKC. The first and second loops were phosphorylated to a lesser extent and the carboxy terminus was poorly phosphorylated. Phosphoserine was the only phosphoamino acid detected. Hence, PKC stimulation blunts cannabinoid activation of K_{ir} current, and CB-1 intracellular domains potentially involved in G protein coupling are PKC substrates.

(Supported by NS01588, NS08174, DGAPA IN203293 and DA09203.)

[1]Koyanao, et al. Eu. J. Neurosci. 5:1189, 1993.

719.6

TYROSINE PHOSPHORYLATION DOWN-REGULATES A CLONED VOLTAGE-GATED K^+ CHANNEL. D.A. Fadool*, K.S. Berman, T.C. Holmes, and I.B. Levitan. Dept. of Biochem. and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

We are studying the regulation of ion channels by protein tyrosine kinases (TKs) using a cloned voltage-dependent potassium channel, Kv1.3, as a model. Kv1.3 channels are studied by transient expression of their cDNAs in human embryonic kidney (HEK 293) cells using lipofectamine transfection. We have demonstrated previously that Kv1.3 tyrosine phosphorylation increases following co-expression with the constitutively active cellular TK, v-src. The functional correlate of such tyrosine phosphorylation was examined by patch-clamping HEK 293 cells in the cell-attached configuration. Kv1.3 transfected cells exhibit a characteristic slowly inactivating macroscopic current in response to a depolarization from -80 to +40 mV; this current is absent in cells co-transfected with Kv1.3 and v-src. However, the Kv1.3 current is observed when co-transfected cells are treated for 7 min to 24 h with either of three different TK inhibitors: 50 μM genestein, 50 μM ST638, or 1.7 μM herbimycin A. Diadzein (59 μM), the inactive structural analog of genestein, does not produce this effect. We have constructed single point mutants (Y to F) of Kv1.3 at putative tyrosine phosphorylation consensus sequences. Mutation of residue 449 near the C-terminus (Y449F) generates a mutant channel that has similar kinetic properties to the wild-type Kv1.3. However, the Y449F mutant channel is not down-regulated by co-transfection with v-src. This suggests that tyrosine phosphorylation down-regulates the Kv1.3 channel, and that residue Y449 is a specific target for TKs.

719.7

EXPRESSION OF THE VOLTAGE-DEPENDENT POTASSIUM CHANNEL Kv1.3 DECREASES CELLULAR TYROSINE PHOSPHORYLATION. T.C. Holmes*, K.S. Berman, M.R. Bowlby and I.B. Levitan. Dept. of Biochemistry and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Tyrosine phosphorylation modulates the properties of the Kv1.3 K^+ channel. To determine whether Kv1.3 activity in turn alters tyrosine phosphorylation, human embryonic kidney (HEK 293) cells were transfected by lipofection with cDNAs coding for control-vector, Kv1.3 and/or the constitutively active v-src tyrosine kinase. The expression of these proteins was verified in cell lysates by western blot analysis using specific antibodies. Protein tyrosine phosphorylation (PYP) in cell lysates was detected by western blot analysis using phosphotyrosine specific monoclonal antibodies. Treatment with the membrane permeant tyrosine phosphatase inhibitor pervanadate (250 μM, 5-60 min) results in robust increases in PYP in control-vector transfected cells. Kv1.3 transfected cells exhibit 40-50% lower PYP following pervanadate treatment as compared to control cells, without any change in the protein levels of cellular kinases. Thus, Kv1.3 expression inhibits the activity of endogenous tyrosine kinases. Similar Kv1.3 induced decreases in PYP are observed in cells co-transfected to express the channel together with v-src. Transfection with a non-conducting mutant (W386F) Kv1.3 channel does not cause a decrease in PYP. Thus potassium channel activity can modulate tyrosine kinase signal transduction pathways.

719.8

THE EGF RECEPTOR TYROSINE KINASE INHIBITS THE VOLTAGE-DEPENDENT POTASSIUM CHANNEL Kv1.3. M.R. Bowlby*, T.C. Holmes and I.B. Levitan. Dept. of Biochem. and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

The epidermal growth factor receptor (EGFr) tyrosine kinase is known to directly tyrosine phosphorylate a variety of targets within cells, as well as couple to several downstream serine/threonine kinase signaling pathways. We have been examining modulation of the voltage-dependent potassium channel, Kv1.3, by the EGFr. cDNA's for the human EGFr and Kv1.3 were expressed in HEK 293 cells using lipofectamine reagent. Expression and tyrosine phosphorylation levels of Kv1.3 and other EGFr substrates were detected using biochemical techniques, while Kv1.3 activity was measured by patch clamp recording. Co-expression of the EGFr and Kv1.3 results in a large increase in the tyrosine phosphorylation of Kv1.3, without affecting its expression. Physiologically, co-expression of the EGFr and Kv1.3 produces a decrease in peak current levels as compared with co-expression of Kv1.3 and vector control. Treatment of EGFr and Kv1.3 co-transfected cells, with an inhibitory EGFr ligand binding domain antibody, blocks the EGFr-induced downregulation of Kv1.3. In addition, acute EGF treatment of co-transfected cells produces a decrease in peak current and some speeding of inactivation kinetics. The physiological modulation may be due to direct tyrosine phosphorylation of the channel, or may occur via downstream signaling events.

719.9

THE *DROSOPHILA SLOWPOKE* (*dSlo*) CALCIUM-DEPENDENT POTASSIUM CHANNEL IS A SUBSTRATE FOR PROTEIN KINASES. J. Wang* and I. B. Levitan. Dept. of Biochem. and Center for Complex Systems, Brandeis University, Waltham, MA 02254.

The activity of the cloned calcium-dependent potassium channel *dSlo* can be modulated by protein phosphorylation (Esguerra *et al*, 1994). To determine whether *dSlo* is a substrate for protein kinases, partial *dSlo* sequences were expressed in *E. coli* as glutathione-S-transferase fusion proteins. Purified fusion proteins were examined for their ability to be phosphorylated *in vitro* by protein kinases. One fusion protein containing *dSlo* residues 821-993 is readily phosphorylated by both protein kinase A catalytic subunit (PKA) and protein kinase C (PKC). In contrast, a corresponding mutant fusion protein in which the serine residue at position 942 (S942), within a consensus PKA site, is replaced by alanine (S942A), cannot be phosphorylated by either kinase under the same assay conditions. These results indicate that S942 can be phosphorylated by both kinases *in vitro*. Another fusion protein containing the last 828 residues (337-1164) of *dSlo*, a proposed cytoplasmic domain of the channel protein, can also be phosphorylated by both kinases. An S942A mutation abolishes the phosphorylation by PKA, suggesting that S942 is the only target for PKA in this long stretch of protein sequence. However, the same mutant protein can still be phosphorylated by PKC, indicating the presence of other serine or threonine residues accesible to PKC.

719.10

REGULATION OF POTASSIUM CHANNEL EXPRESSION IN DEVELOPING GnRH NEURONS BY FIBROBLAST CONTACT. Martha M. Bosma*. Dept. Pharmacology, Univ. Washington, Seattle WA 98195.

GnRH neurons in the adult animal are situated in the hypothalamus, but are embryonically derived from the peripheral olfactory placode, making them a unique system in which to study CNS neurons during migration and development. GnRH neurons were enzymatically dissociated from olfactory placodes isolated from E11.5 mouse embryos, currents recorded using whole cell patch clamp, and cells identified by immunocytochemistry. Isolated GnRH cells were compared with those in contact with fibroblasts. Isolated GnRH cells increased in size (measured by cell capacitance) with time in culture from 2.8±0.2 pF (n=18) on day 1 to 11.8±2.7 pF (n=4) on day 5. From days 1-5 peak outward current increased, but current density remained low due to the increase in cell size (day 1, 10.6±1.8 pA/pF; day 5, 6.3±1.8 pA/pF). 50% of these isolated cells expressed a small transient inward current. In contacted cells, outward currents were larger on day 2 (15.8±2.7 pA/pF, capacitance 6.6±1.3 pF, n=5), and increased on days 3-5 (day 5, 35.5±9.1 pA/pF, capacitance 6.5±0.6, n=4). In addition, these cells expressed a large transient inward current. Immunocytochemistry with an antibody specific to the Shaker subfamily potassium channel mKv1.1 demonstrated expression of this protein in the isolated GnRH neurons. Supported by the Andrew W. Mellon Foundation.

719.11

EFFECT OF DOCOSAHEXAENOIC ACID ON $mK_V1.1$ AND $mK_V1.2$ K^+ CHANNELS. Jennifer C. Garratt, Matthew P. M^c Evoy and David G. Owen* Molecular Pharmacology, Wyeth Research, Taplow, Berkshire, SL6 0PH, UK.

Docosahexaenoic acid (DOHA) is a long chain polyunsaturated fatty acid which is abundant in fish oil. External administration of DOHA caused a time dependent block of the cardiac channel, $K_V1.5$ (Honoré *et al*, 1994). Using whole-cell patch clamp, the potassium channel-blocking effects of DOHA were examined on two cloned mouse brain potassium channels, $mK_V1.1$ and $mK_V1.2$ stably expressed in Chinese hamster ovary cells. Transfected cells expressed a non-inactivating, "delayed rectifier"-type potassium channel; $mK_V1.2$ has a slower activation rate. For purposes of quantifying dose-dependent block of these currents overall charge transferred during the voltage step (Q_{step}) was measured. External administration of DOHA produced a dose-dependent, reversible block of $mK_V1.1$ (EC_{50}=7.7μM) and $mK_V1.2$ (EC_{50}=2.1μM). Blockade of these channels is time-dependent *eg* at +70 mV for $mK_V1.1$ DOHA (15μM) the time constant of deactivation (τ_D) was 52.5±4.5ms and for $mK_V1.2$ DOHA (10μM) was 11.6± 0.34ms. The time constants of the DOHA induced decay of $mK_V1.1$ and $mK_V1.2$ decreased in a concentration-dependent fashion. Interestingly DOHA was found to increase the rate of activation of $mK_V1.2$ (0.86±0.02ms compared with control value 1.78±0.14ms). Internal administration of DOHA (300μM) did not alter either $mK_V1.1$ or $mK_V1.2$ current. These findings suggest that DOHA blocks both $mK_V1.1$ and $mK_V1.2$ channels at a site which is only accessible via the external mouth of the channel and only once the channel is open.

719.12

THE EFFECTS OF THREE DENDROTOXINS AND 4-AMINOPYRIDINE ON AN ENDOGENOUS OUTWARD CURRENT AND A HETEROLOGOUSLY-EXPRESSED POTASSIUM CHANNEL(rKv1.1) IN HEK-293 CELLS. F. Heblich, M. Minchin* and D. G. Owen. Electrophysiology Laboratory, Molecular Pharmacology Department, Wyeth Research (UK), Huntercombe Lane South, Taplow, Berks, SL6 0PH.

Using the whole cell patch clamp technique we have examined the electrophysiological characteristics of the cloned potassium channel, rKv1.1 (RCK1), stably-expressed in HEK-293 cells. The steady-state conductance-voltage relationship for rKv1.1-expressing cells was best fitted with a two component Boltzmann function, consistent with the presence of an endogenous current in the HEK-293 cells. Examination of untransfected cells revealed in many cases, an endogenous outward current, activated by depolarization with amplitudes of up to ca. 600pA (measured at +45mV; V_H = -90mV). The conductance-voltage relationship of the endogenous current was fitted by a single component Boltzmann function, with a slope equivalent to that of the second component of the conductance curve obtained in transfected cells. 4-aminopyridine (4-AP) and three dendrotoxin (Dtx) homologues, toxin K, α- and δ-Dtx blocked rKv1.1 currents in a dose-dependent manner. IC_{50}s were 195μM, 241pM, 23nM and 130pM, respectively, compared to IC_{50}s of 2.5nM for toxin K (Robertson *et al*, 1994. *J. Physiol.*, **481**(P), 41P) and 5nM for α- and 1nM for δ-Dtx (Wittka & Pongs personal communication) *vs* rKv1.1 when expressed in oocytes. Whereas, high concentrations of 4-AP and toxin K blocked all voltage-activated current α and δ-Dtx blocked ca. 80% max. All of the blockers caused a two-fold slowing of the activation time constant (τ_n) of rKv1.1 (e.g. 10nM δ-Dtx lengthened τ_n from 0.95±0.15ms to 2.5±0.35ms at +45mV ,n=4). It could therefore be suggested that toxin K, α-Dtx, δ-Dtx and 4-AP hinder the allosteric changes which occur as the channel opens. Thanks to Prof. O. Pongs for the rKv1.1 cell-line and Dr. B. Robertson for helpful discussions.

719.13

STUDIES ON THE N-TYPE INACTIVATION OF THE MOUSE Kv1.1 CHANNEL INDUCED BY THE Kv3.4 BALL PEPTIDE. G. J. Stephens, D. G. Owen, A. Opalko, M. R. Pisano, W. H. McGregor & B. Robertson*. Wyeth Research (U.K), Taplow, Berkshire SL6 0PH, U.K. and Wyeth-Ayerst, Radnor, PA 19101, U.S.A.

A 28-mer peptide based on part of the N-terminal of the human Kv3.4 K^+ channel (NleISSVCVSSYRGRKSGNKPPSKTCLKEE) transforms the mouse brain cloned K^+ channel, mKv1.1, from a non inactivating to a rapidly inactivating current. Using the whole cell patch clamp technique to examine this 'ball and receptor' model system in Chinese hamster ovary cells, we have probed some of the residues important for N-type inactivation by introducing various mutations and deletions to the hKv3.4 peptide. The sensitivity of hKv3.4 peptide to redox modulation is critically dependent on the cysteine at position 6 (Stephens & Robertson; *J. Physiol.* 484, 1-13). Cysteine to serine substitutions destabilised the inactivated state of the mKv1.1/3.4 peptide channel, but did not abolish inactivation. Control time constant of recovery from inactivation ($\tau_{rec.}$) at -90 mV was 1.3 ± 0.1 s (n=9); this was faster in the mutants C6S (0.2 ± 0.04 s; n=3), C24S (0.6 ± 0.1 s; n=4) and C6S, C24S (0.3 ± 0.02 s; n=7). The apparent K_d (control = 8 ± 2 μM; n=7) was most drastically reduced by the double mutant C6S, C24S (44 ± 9 μM; n=5) and C24S (22 ± 8 μM; n=6), but not by C6S (12 ± 5 μM; n=4). Deletion of the five residues preceding C6 (Δ1–5) reduced the apparent affinity of the peptide (K_d = 33 ± 12 μM; n=7); this deletion most noticeably affected association rate (K_{on}), with the control value of 0.31 ± 0.05 $x10^6M^{-1}s^{-1}$ (n=7) being reduced to 0.05 ± 0.01 $x10^6M^{-1}s^{-1}$ (n=4) for Δ1–5. In contrast, deletion of the four residues after C24 (Δ25–28) caused an increase in K_{on} to 0.78 ± 0.16 $x10^6M^{-1}s^{-1}$ (n=8); Δ25–28 had a K_d of 6 ± 2 μM (n=8). The results indicate that neither cysteine residue is essential for N-type inactivation, but that their presence does stabilise the inactivated state and that residues closest to the N-terminal may be important for occupation of the ball receptor. It is hoped that such studies will reveal more about the processes of inactivation, and possibly its potential for modulation.

719.14

ACCESS OF SUBSTANCES TO POTASSIUM CHANNELS IN XENOPUS OOCYTES: INFLUENCE OF FOLLICULAR TISSUES. M. Madeja*, U. Mußhoff and E.-J. Speckmann. Institut für Physiologie, Robert-Koch-Str. 27a, D-48149 Münster, Germany

Oocytes of Xenopus laevis are a widespread model system to express and characterise neuronal ion channels and receptors. Concerning the investigations of these molecules, however, it is unclear, if the oocytes' follicular tissues reduce the access of substances to the molecules expressed. In order to shed some light on this, the effects of various blocking agents on currents of voltage-operated Kv1.1 potassium channels were studied in oocytes with and without follicular tissues.

The experiments revealed the following: (i) Each tested blocking agent was more effective when applied to oocytes without follicular tissues. This was found true for ions (lead, barium), smaller molecules (TEA, 4-AP, diltiazem, nifedipine, verapamil, pentylenetetrazol) and large molecules (DTX, MCDP; n = 9 to 21 each). (ii) The concentration-response curves were shifted to the left in oocytes without follicular tissues. The IC_{50} values in oocytes with and without tissues were 4.1 and 1.0 mmol/l for TEA and 30.0 and 1.2 μmol/l for lead, respectively. (iii) The times needed to reach the half-maximal blocking effect (t_{50}) were reduced in oocytes without follicular tissues. The t_{50} values in oocytes with and without tissues were 0.33 and 0.07 s for TEA, 8.8 and 2.4 s for DTX and 4.2 and 0.2 s for lead, respectively.

On a whole, the follicular tissues appear to reduce the sensitivity of the potassium channels to blocking agents. This 'barrier effect' has to be considered in pharmacological work on oocytes.

719.15

BK_{CA}-CHANNEL PHARMACOLOGY: A COMPARISON BETWEEN CLONAL AND RECOMBINANT CELL LINES

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Ca^{2+}-regulated potassium (K)-channels of large conductance (BK_{Ca}-channels) are widely distributed in peripheral tissues as well as in the central nervous system (CNS) and are also expressed in a variety of neural cell lines including PC-12, NG108-15, C6-BU1 and GH3 cells. The BK_{Ca}-channels from drosophila (dslo), mouse (mslo, 1) and human brain (2) have recently been cloned and stably expressed in CHO cells. The aim of the present study was to compare the pharmacological profiles of BK_{Ca}-channels expressed in rat glioma C6-BU1 and pituitary GH3 cells with those in recombinant CHO cells transfected with dslo (PTS2, from O. Pongs) or mslo (from L. Salkoff) by means of rubidium (Rb) efflux. For a number of BK_{Ca}-channel blockers including charybdotoxin, iberiotoxin, tetrandrine, quinine and the dihydropyridines nitrendipine, nicardipine and nimodipine good and signifincant correlations of IC_{50}-values from inhibition of ionomycin-stimulated Rb-efflux from C6-BU1, PTS2 and mslo cells were obtained. However, the potencies of the compounds were generally somewhat lower in the recombinant cells, especially the toxins were up to 30-fold less active. The indole alkaloid paspalicine, reported to block BK_{Ca}-channels (3) did not inhibit the Rb-efflux from either cells. Moreover, in patch clamp recordings from GH3-cells using either outside-out or inside-out patches this compound did not significantly block single BK_{Ca}-channels. The present data reveal very similar, if not identical, pharmacological profiles of BK_{Ca}-channels of C6-BU1, GH3 and recombinant cells.

1. Butler et al., Science ,261, 221 (1993)
2. Dworetzky et al., Mol.Brain Research, 27, 189 (1994)
3. Knaus et al., Biochemistry, 33, 5819, (1994)

POTASSIUM CHANNEL PHYSIOLOGY, PHARMACOLOGY AND MODULATION IV

720.1

CHARACTERIZATION OF [^{125}I]-α-DENDROTOXIN BINDING TO A STABLE CELL LINE EXPRESSING THE RAT VOLTAGE-DEPENDENT POTASSIUM CHANNEL Kv1.2

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Alpha-Dendrotoxin (α-DTX) is a potent blocker of potassium (K+) currents associated with Kv1.1, Kv1.2 and Kv1.6 voltage-gated K+ channels. The rank order of potency determined by electrophysiological technique in clonal cell lines or oocytes for α-DTX is : Kv1.2 > Kv1.1 > Kv1.6. Furthermore, biochemical studies have revealed that Kv1.2 is the major component of the DTX-sensitive receptors in brain. The present study characterized the radioligand binding profile of [^{125}I]-α-DTX to the rat Kv1.2 subunit stably expressed as a homomeric channel. The cDNA for Kv1.2 (RAK) was subcloned into a mammalian expression vector (pREP9) containing a neomycin resistance gene. Subsequently, the plasmid was transfected using lipofectamine into LMTK$^-$ cells which were grown in the presence of neomycin to select for cells expressing the Kv1.2 subunit. Individual clones were initially screened for expression of Kv1.2 by immunocytochemistry using an affinity-purified polyclonal antibody to the rat Kv1.2 subunit and later confirmed by [^{125}I]-α-DTX radioligand binding. [^{125}I]-α-DTX binding to whole cells expressing the rat Kv1.2 channel was saturable and of high affinity with a calculated Kd and Bmax of 128 ± 17 pM and 33 ± 9.9 fmol/mg protein, respectively. The rank order of potencies for K+ channel blockers was Margatoxin (0.66 ± 0.12 pM) > Stichodactyla Toxin (30 ± 11 pM) > α-DTX (24 ± 8 pM) ≈ Charybdotoxin (CTX;23 ± 3 pM) > Mast Cell Degranulating Peptide (588 ± 86 pM). Interestingly, the Hill coefficient for CTX was significantly closer to unity in the Kv1.2 cell line as compared to previous results in rat brain. Although Kv1.2 plays an important role as a DTX-sensitive subunit in brain, the pharmacological profile of [^{125}I]-α-DTX binding differs significantly between brain and the clonal Kv1.2 cell line.

720.2

BEHAVIORAL EFFECTS OF THE POTASSIUM CHANNEL BLOCKER 4-AMINOPYRIDINE AND ITS STRUCTURAL ANALOGS

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Biochemical and electrophysiological studies have demonstrated that 4-aminopyridine (4-AP) and its structural analogs are blockers of voltage-dependent potassium (K) channels. However, few studies have evaluated the behavioral effects of this group of compounds and several studies have suggested that many of the analogs have limited CNS penetrability. In the present study, rats (N=6) were trained to discriminate 4-AP (1 mg/kg s.c) from saline in a two-lever drug discrimination procedure. 4-AP (0.3-1.7 mg/kg) produced dose dependent increases in responding on the drug appropriate lever with moderate rate decreasing effects at the highest dose. After the discrimination was established, substitution tests were conducted with the structurally related analogs 2-AP, 3-AP, 3,4-diaminopyridine (3,4-DAP), 2,6-DAP, and pyridine HCl. Like 4-AP, 3-AP (1-10 mg/kg) and 2,6-DAP (0.3-17.8 mg/kg) substituted fully in all animals tested. 2-AP (1-30 mg/kg) substituted fully in 4 of 5 rats and 3,4-DAP (3-17.8 mg/kg) substituted fully in 3 of 6 rats. Pyridine (3-100 mg/kg) only substituted fully in 1 of 5 rats. In general, doses of the aminopyridines that substituted for 4-AP had little effect on response rates. The rank order of potency and efficacy for substitution was 4-AP>3-AP>3,4-DAP≥2-AP>>pyridine. 4-AP and its analogs including 2,3-DAP and 4-dimethylaminopyridine (4-DMAP) were also evaluated for their convulsant effects in mice. With the exception of pyridine, all compounds produced dose-dependent increases in the percentage of mice exhibiting clonic convulsions. The rank order of potency for producing convulsions was 4-AP>2,3-DAP=3,4-DAP>3-AP≥4-DMAP>2-AP>2,6-DAP>>pyridine. The present results demonstrate that 4-AP and its analogs have similar behavioral effects in rodents that are likely due to their actions at CNS K-channels.

720.3

ANTIDEPRESSANT-LIKE EFFECTS OF POTASSIUM CHANNEL BLOCKERS

T.M.K. Trella*, S. Thomas, A. Thurman, J.E. Barrett and S. Rosenzweig-Lipson. Wyeth-Ayerst Research, CNS Biology, Pearl River, NY 10965.

The antidepressant-like effects of the potassium channel blockers 4-aminopyridine (4-AP) and quinine were compared with those of imipramine in three animal models (tail suspension test, forced swim test, DRL-72 sec schedule) that are sensitive to clinically effective antidepressants. For the tail suspension test, mice were suspended upside down by their tails and the duration of immobility was scored during a 6-min test session. For the forced swim test, rats were placed in a cylinder of water for a 15-min swim pretest. A subchronic dosing procedure was used, with injections occuring 30 min, 19 hours and 23 hours following the swim pretest. At the 24th hour, rats were placed in a cylinder of water and the duration of immobility was scored during a 5-min swim test session. For the DRL 72-sec schedule, rats were trained to lever press for a food pellet reinforcer. A response was reinforced only if it occurred 72-sec after the previous response or the start of the session. Both 4-AP and imipramine produced antidepressant-like effects in all three animal models. 4-AP, like imipramine, produced dose-dependent decreases in immobility time in the forced swim and tail suspension tests. In addition, both 4-AP and imipramine dose-dependently increased the number of reinforcers earned and produced modest decreases in response rate in rats responding under a DRL 72-sec schedule. Quinine produced antidepressant-like effects in two of three animal models. Quinine produced dose-dependent decreases in immobility time in the forced swim test, but did not affect immobility time in the tail suspension test. Quinine also produced dose-dependent increases in the number of reinforcers earned and produced modest decreases in response rate in rats responding under a DRL 72-sec schedule. The present results suggest that K-channel blockers may have therapeutic potential as antidepressant drugs.

720.4

5-HT_{2A} RECEPTOR INVOLVEMENT IN THE EFFECTS OF THE POTASSIUM CHANNEL BLOCKER 4-AMINOPYRIDINE.

A.L. Gorman*, S. Rosenzweig-Lipson and L.E. Schechter. Wyeth-Ayerst Research, CNS Disorders, Pearl River, NY 10965.

Previous studies have shown that the potassium (K^+) channel blocker 4-aminopyridine (4-AP) induces wet dog shakes, forelimb and head movements, and tremors in rats. Since these behaviors resemble those produced by 5-HT_{2A} agonists, the present studies were conducted to determine whether 4-AP-induced behavioral changes were mediated by 5-HT_{2A} receptors. In the first study, rats were pretreated with the 5-HT_{2A} receptor antagonist ketanserin (3 mg/kg ip) 30 min prior to administration of 4-AP (1 or 3 mg/kg sc) or saline (S). During the 30 min post-injection period, 4-AP increased the number of wet dog shakes (Mean±SEM: S=0, 1 mg/kg=19±6, 3 mg/kg=14±5) and the number of rapid repetitive forelimb movements (S=0, 1 mg/kg=19±8, 3 mg/kg=14±8). Ketanserin pretreatment attenuated both 4-AP-induced effects (Wet dog shakes:S=0, 1 mg/kg=4±3, 3 mg/kg=6±2; Repetitive forelimb movements:S=0, 1 mg/kg=2±1, 3 mg/kg=6±4), suggesting 5-HT_{2A} receptor modulation of these behavioral responses. To characterize further the pharmacological properties of 4-AP, rats were injected with either 4-AP (1 mg/kg sc) or saline once daily for 3 weeks, and sacrificed 24 hours after the last injection for tissue collection. In whole cortical membrane preparations, 5-HT_{2A} receptors were labeled with [^{3}H]ketanserin (0.15-5 nM), and non-specific binding was defined by 1 μM cinanserin. A significant decrease in Bmax was detected in the cortex of rats chronically treated with 4-AP (Mean±SEM (fmol/mg): saline=306±57; 4-AP=157±30), with no significant changes in K_d noted. Therefore, chronic 4-AP treatment resulted in a down-regulation of 5-HT_{2A} receptors, an effect associated with antidepressants (see Trella, this meeting). The results indicate that 4-AP-induced effects may be partially mediated by 5-HT_{2A} receptors. These data are consistent with the *in vitro* results of Schechter (this meeting) who found that 4-AP stimulates [^{3}H]5-HT release. Taken together, the results suggest that K+ channel blockade modulates serotonergic neurotransmission.

720.5

A Novel Neuroselective Agent Producing Glibenclamide-insensitive, K+-dependent Hyperpolarization. J.C. Chisholm*, J.N. Davis and E.J. Hunnicutt, Jr., Institute for Dementia Research, Bayer Corp., West Haven, CT 06516

Although the widespread presence and diversity of K+ channels make them a potential site of selectivity for pharmacologic intervention, there are far more classes of K^+ channel characterized than there are pharmacological agents known to interact with them. We reported a novel hyperpolarizing activity found in BAY x 9227, an enantiomer of a K^+_{ATP} channel activator, which differed from the latter in important ways: it is remarkably potent (EC_{50} 3 picomolar), with greater than 1000-fold selectivity for neurotypic over vascular smooth muscle cells, and is not blocked by the K^+_{ATP} channel blocker, glibenclamide [Eur. J. Pharm. 261:R1 (1994)]. We now report that one strain of N1E-115 neuroblastoma cells, which was unresponsive to K^+_{ATP} agents and showed no hyperpolarization in response to ATP depletion (produced by application of oligimycin and 2-deoxy-D-glucose), responded with hyperpolarization to 30 pM BAY x 9227. Hyperpolarizing activity is eliminated by removing the electrochemical driving force for K^+ out of the cell. Finally, functional correlates to the hyperpolarization produced by BAY x 9227 include inhibition of depolarization-induced elevations in neuronal $[Ca^{2+}]_i$, protection from glycine(Mg^{2+}-free)-induced neurotoxicity and inhibition of depolarization-induced insulin release from RINm5F cells, all produced by concentrations of BAY x 9227 less than 30 pM. In combination, these data implicate a site other than classic K^+_{ATP} channels for mediation of the potent effects of this compound, and suggest a novel site for therapeutic intervention.

720.6

A BASELINE CHANNEL IN *APLYSIA* NEURONS IS INHIBITED BY FMRFAMIDE AND HALOTHANE. Bruce D. Winegar*, John R. Forsayeth, and C. Spencer Yost. Department of Anesthesia, University of California, San Francisco, San Francisco, CA 94143-0648.

We have studied volatile anesthetic actions on single noninactivating ion channels in the nervous system of the marine mollusk, *Aplysia californica*. Outward single-channel currents were recorded from pleural ganglion neurons with artificial seawater in the recording pipette. Several distinct baseline channels were observed, including a voltage-insensitive, noninactivating channel with a linear slope conductance of ~40 pS that reversed at ~-40 mV. The channel was active in cell-attached and excised patches and was insensitive to external 10 mM TEA. Channel activity was completely inhibited in the presence of 3 μM FMRFamide (Phe-Met-Arg-Phe-NH_2). A reversible decrease in the channel mean open probability was produced by perfusion with 400 μM (0.008 atm) halothane, which shortened the open times but did not affect the current amplitudes. Our results demonstrate that a distinct baseline channel is inhibited by clinical concentrations of volatile anesthetics. Baseline channels contribute to the resting membrane potential and may be possible target sites that mediate volatile anesthetic actions.

720.7

ORGANIC AMINE BLOCKADE OF DOPAMINE-MODULATED K^+ CHANNELS ON RAT CAUDATE-PUTAMEN NEURONS. Yong-Jian Lin* and Jonathan E. Freedman. Dept. Pharmaceutical Sciences, Northeastern Univ., Boston, MA 02115.

Using cell-attached patch-clamp recording from freshly dissociated rat corpus striatum neurons, we are studying the blockade properties of an inwardly-rectifying 85 pS K^+ channel, which is activated by D_2-like dopamine receptors. Previous studies showed that this channel was sensitive to sulfonylurea drugs, and was blocked by nanomolar concentrations of quinine and related quinuclidine amines. Here, we found that both tetraethylammonium (TEA, 5 mM) and 4-aminopyridine (4-AP, 1 mM) partially blocked this channel when applied to the external surface of the membrane via the patch pipette. Block was relatively voltage-insensitive, and was accompanied by a reduction of open time and an increase of closed time. These cells also express other inwardly-rectifying K^+-permeable channels with conductances ≤35 pS, which are dopamine-insensitive. In contrast to the 85 pS channel, these channels were largely insensitive to block by 10 mM TEA or 5 mM 4-AP. Our results indicate that the dopamine-modulated K^+ channel is blocked by various organic amines with differing potencies, and that inwardly rectifying ion channels of the striatum are heterogeneous in their sensitivities to blockade by these compounds.

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720.8

MECHANISMS OF DIHYDROPYRIDINE BLOCK OF SHAKER POTASSIUM CHANNELS. A. Kamath, K. Larson, E.F. Shibata*, and T. Hoshi, Dept. of Physiology and Biophysics, Univ. of Iowa, Iowa City, Iowa 52242.

Dihydropyridines (DHPs) are well known as Ca^{2+} channel agonists and antagonists. Recent evidence indicates that DHPs also affect voltage-dependent K^+ channels. Thus, we examined how DHPs affect *Shaker* K^+ channels expressed in Xenopus oocytes using the patch-clamp method.

Nifedipine, nicardipine, nimodipine, and Bay K 8644 at 10 to 100 μM induced a time-dependent reduction of the K^+ currents of mutant Shaker channels with disrupted N- and C-type inactivation (ShBΔ6-46 T449V). The time course of the current decline induced by the DHPs was approximated by one exponential. When applied intracellularly, the onset of the current reduction was rapid and the effect was reversible. Single channel analysis showed that the DHPs reduced the mean open time in a concentration-dependent manner without affecting unitary conductance.

Efficacy of the DHPs was dependent on residue 463 located in the S6 segment. This residue has been shown to influence C-type inactivation. The estimated DHP off rate was much slower with isoleucine at this position than with alanine. Since the DHPs induced an apparent inactivation, we investigated whether they compete with N- or C-type inactivation. The results suggest that the DHPs do not compete with either inactivation and that they may work to accelerate the intrinsic inactivation rates.

Structural groups on different DHPs crucial in reducing K^+ channel currents were also examined. The estimated off rate was greater for nifedipine and Bay K 8644 than for nicardipine, suggesting that the side group at C3 may be important in determining the DHP efficacy. (Supported by American Heart Association Iowa Affiliate, and Klingenstein Fellowship to T.H. and an Established Investigatorship award from the AHA to E.F.S.)

720.9

PROSTACYCLIN ANALOG CARBACYCLIN INHIBITS CA^{2+}-ACTIVATED K^+ CURRENT IN CULTURED BARORECEPTOR NEURONS. Z. Li, H. Lee, K. Bielefeldt, M.W. Chapleau,* and F.M. Abboud. Depts. of Physiol. & Biophys. and Internal Med., Univ. of Iowa Coll. of Med. and DVA Med. Ctr., Iowa City, IA 52242.

In the isolated carotid sinus prostacyclin enhances arterial baroreceptor (BR) sensitivity. We tested the hypothesis that the mechanism involves inhibition of K^+ currents. BR neurons from rat nodose ganglia were dissociated and identified using retrograde label with fluorescent dye (DiI) applied to the aortic arch two weeks earlier. Whole-cell outward K^+ current was recorded with standard patch-clamp techniques and measured at 200 ms after depolarization from -40 to +40 mV. Drugs were delivered by exchanging the bath solution. The stable prostacyclin analog carbacyclin (CC; 100μM) reversibly inhibited the current to 65±6% of control (n=8). CC failed to inhibit the current in the absence of Ca^{2+} in pipette and bath solutions (n=4). Charybdotoxin (CTX; 100nM), a specific blocker of Ca^{2+}-activated K^+ current ($I_{K\text{-}Ca}$), inhibited the outward current (73±3% of control). CC did not cause any further inhibition after CTX (n=6). Including $PKI_{(5\text{-}24)}$, a specific inhibitor of protein kinase A, in the pipette solution abolished the inhibitory effect of CC (n=6). We conclude that prostacyclin inhibits the CTX-sensitive $I_{K\text{-}Ca}$ in BR neurons through a protein kinase A-dependent pathway. This mechanism may contribute to the sensitization of BR by prostacyclin.

720.10

NITRIC OXIDE ACTIVATES CALCIUM-DEPENDENT POTASSIUM CHANNELS IN CEREBROVASCULAR SMOOTH MUSCLE CELLS. C. Chen, D.A. Mathers*. Dept. of Physiology, Faculty of Medicine, Univ. of B.C., Vancouver, B.C., Canada, V6T 1Z3

Nitric oxide is probably the major endothelium-derived relaxing factor (EDRF). It is thought to relax smooth muscle cells by stimulation of guanylate cyclase, accumulation of its product cyclic GMP, and cGMP-dependent modification of several intracellular processes, including activation of potassium channels through cGMP-dependent protein kinase. Here we present evidence that sodium nitroprusside (SNP), a vasodilator that decomposes into nitric oxide, can directly activate large conductance calcium-dependent potassium channels (K_{Ca} channels) in cell-free, inside-out membrane patches excised from cerebrovascular smooth muscle cells of adult rats.

The K_{Ca} channels studied here showed the conductance of 203±8.3pS (mean ±s.e, n=15) between -80 and +80 mV in symmetrical 140 mM KCl solutions. Bath solution contained (in mM): 140 KCl; 2.86 $CaCl_2$; 10 HEPES; 3 EGTA (free calcium concentration 1μM). Patch pipettes solution contained (in mM): 140 KCl; 1.48 $CaCl_2$; 10 HEPES; 3 EGTA. Application of SNP (100 μM) to the bath solution enhanced the K_{Ca} channel open probability by 1.6±0.12 times (mean±s.e., n=15), particularly the mean closed time of the channels was 0.6±0.05-fold (mean ±s.e., n=15) that seen in control solution. However, no difference was found between the conductances of SNP activated and control K_{Ca} channels.

720.11

REDOX AND NITRIC OXIDE (NO)-DEPENDENT MODULATION OF POTASSIUM CHANNELS OF SQUID STELLATE GANGLION NEURONS. [1]W.F. Gilly, L.L. Moroz and R. Gillette*. [1]Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950; Dept. Physiology, University of Illinois, Urbana/Champaign, IL 61801.

Stellate ganglia (SG) are the main peripheral centers controlling coordinated mantle contractions and jet propulsion in cephalopod molluscs. In many species the SG giant fibre lobe (GFL) neurons fuse to form giant motor axons. We have screened for distribution of putative NO synthase containing cells in SG of cuttlefish (*Sepia officinalis, Rossia pacifica*) and squid (*Loligo opalescens*) NADPH-diaphorase (NADPH-d) histochemistry. Both *Sepia* and *Rossia* had either no or very low levels of NADPH-d activity in SG. In contrast, intense NADPH-d labelling was detected in *Loligo* around neuronal somata, mainly in the medial part of SG and in the sheath of the stellate nerve axons (including giant axons). This suggests that NO might modulate neuronal activity in squid SG. In experiments on isolated, cultured GFL neurons we found that NO-donors (SNAP, DEA) increase the rate and extent of inactivation of K-current in whole cell patch recordings. The reducing agent DTT potentiated these effects when applied extracellulary with the NO-donors. These effects were also seen in insect Sf-9 cells in which the major K-channel gene expressed by GFL neurons, squid Kv1.1 (previously called KZ4, Perry et al., 1995 Biophys. J., 66,:A105), was expressed via baculovirus infection. The action of NO-donors was not mimicked by 8-Br-cGMP and may arise from direct modulation of K-channel proteins.

720.12

LINOPIRDINE, A NEUROTRANSMITTER RELEASE ENHANCER, BLOCKS VOLTAGE-ACTIVATED POTASSIUM CURRENTS IN HIPPOCAMPAL NEURONS. M.E. Schnee and B.S. Brown*, Preclinical Pharmacology, The DuPont Merck Pharmaceutical Co., Wilmington, DE 19880.

Linopirdine [3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one] enhances the potassium-stimulated release of a number of neurotransmitters from rat neocortex, hippocampus and striatum *in vitro* without affecting basal release. Recently linopirdine was found to block M-current in hippocampal CA1 neurons with an IC_{50} of 8.5 μM, a value closely paralleling it's IC_{50} for enhancement of acetylcholine release (3.2 μM). To determine whether linopirdine selectively blocks M-current, whole cell and outside-out patch clamp techniques were used to examine its effects on other voltage-activated potassium currents of CA1 neurons in rat hippocampal slices.

In whole cell experiments, the peak amplitude of the transient (I_A) and sustained (I_K) outward currents were reduced by bath application of linopirdine in a dose dependent manner with an IC_{50} of approximately 100 and >300 μM, respectively. Linopirdine also markedly increased the rate of I_A inactivation. The mixed Na/K inward rectifier, I_Q, and I_{AHP} were unaffected by up to 100 μM linopirdine. In outside-out patch experiments on I_A, the IC_{50} was approximately 30 μM for enhancing the rate of current inactivation and >30 μM for reducing peak current amplitude.

These results support the hypothesis that linopirdine is selective for M-current at concentrations <10 μM. At 10 μM and above the increased rate of I_A inactivation could also contribute to enhanced neurotransmitter release.

720.13

SR141716A BLOCKS CANNABINOID MODULATION OF POTASSIUM A-CURRENT IN CULTURED RAT HIPPOCAMPAL NEURONS

Jian Mu, Robert E. Hampson, Shouyuan Zhuang, Virginia C. King and Sam A. Deadwyler*, Dept. of Physiology & Pharmacology, and Center for the Neurobiological Investigation of Drug Abuse, Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27157.

The potent cannabinoid agonist, WIN 55,212-2, modulates the voltage-dependence of potassium A-current (I_A) in rat hippocampal neurons via a cAMP dependent process (Deadwyler *et al.* 1995, JPET v.275 in press). An antagonist to the CB1 cannabinoid receptor, SR141716A (Sanofi) has been shown to be effective in blocking the reduction in cAMP produced by cannabinoid receptor agonists.

Whole-cell currents were recorded from cultured E-18 fetal rat hippocampal neurons, 7-15 days in culture, with TTX 1μM, 4-AP 500μM and TEA 30mM in the bathing medium to block sodium and non-A-type potassium currents. WIN 55,212-2 (Research Biochemicals) was diluted from 1mM stock in ethanol (which was later evaporated under a nitrogen stream) and delivered via pressure pipette in bathing medium at 1-2μM concentrations. SR141716A was prepared from 1mM stock (in ethanol), diluted to 100-500nM in bathing medium, and applied via bath perfusion.

Pressure pipette application of 1μM WIN 55,212-2 produced a 10-15 mV positive shift in voltage dependence of steady state inactivation that was completely blocked by bath perfusion of 300nM SR141716A. The 15-20 mV positive shift in steady-state inactivation of I_A produced by 2μM WIN 55,212-2 was partially blocked (50%) following 5 min bath perfusion with 300nM SR141716A. Dose effect curves revealed that the EC_{50} for the WIN modulation of I_A was shifted from 0.8μM to 2.0μM via competitive antagonism with SR141716A. Higher concentrations (500nM) of SR141716A antagonist alone precipitated decreases in I_A suggesting the possible presence of an endogenous cannabinoid ligand in the hippocampal cultures.

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720.14

A NEW ASPECT OF AN APOVINCAMIC ACID DERIVATIVE, VA-045, AS A K^+ CHANNEL OPENER IN CNS NEURONS. N. Harata*, M. Munakta, H. Araki** and N. Akaike. *Dept. of Physiology, Kyushu Univ. Fac. of Med., Fukuoka 812-82, Japan, **Research Center, Taisho Pharmaceutical Co. Ltd., Ohmiya 330, Japan.

VA-045 ((+) - Eburnamenine - 14 carboxylic acid (2-nitroxyethyl) ester) is a novel apovincamic acid derivative, which has multiple activities on the central nervous system (CNS). This compound improves neurological disturbances evoked by head injury *in vivo*. However, the mechanism of the action has been largely unknown. We investigated, therefore, the effects of VA-045 on the neurons acutely dissociated from the rat cortex, using nystatin perforated-patch clamp technique. Under the current-clamp mode, VA-045 hyperpolarized the membrane, decreasing the firing activities. Under the voltage-clamp mode (V_H=-40 mV), it evoked an outward current in a concentration-dependent manner (EC_{50}=0.5 μM). The VA-045-induced current resulted from an increased K^+ conductance and had an outward rectification. This current showed distinct pharmacological properties to K^+ channel blockers. Glybenclamide (10μM), an ATP-sensitive K^+ channel blocker, had no effect. Apamin (1μM), charybdotoxin (1μM) or Ba^{2+} (3mM) also failed to block the current, while TEA decreased it (IC_{50}=2mM). VA-045 could repetitively activate the K^+ current in the absence of external Ca^{2+}. Both ryanodine and thapsigargin had no effect on this current. The current was not significantly affected by staurosporine (0.1μM), quinacrine (10μM), wortmannin (1μM) and genistein (10μM). These results indicate that VA-045 may directly activate K^+ channels, not by any of Ca^{2+} or other second messengers. The hyperpolarizing action of VA-045 may protect CNS neurons from overexcitation and extensive Ca^{2+} influx through voltage dependent Ca^{2+} channels in the pathological conditions such as hypoxia and injury.

720.15

TOLBUTAMIDE SUPPRESSES SEVERAL VOLTAGE-DEPENDENT K CURRENTS OF CA1 HIPPOCAMPAL NEURONS **B. Esplin*, G. Erdemli & K. Krnjević, Anaesthesia Research & Pharmacology Departments, McGill University, H3G 1Y6 Montréal, Québec, Canada.**

Though consistently effective as ATP-sensitive K-channel (K_{ATP}) antagonist (Ashcroft & Ashcroft, 1992 Biochim. Biophys. Acta 1175:45), tolbutamide (TOLB) is not wholly selective: it inhibits protein kinases (Kanamori et al 1976 Biochim. Biophys. Acta 429:147) and D-type K channels (Crépel et al 1993 J. Physiol. 466:39). We tested TOLB on voltage-activated outward currents (I_{out}'s) in slices kept at 33° (from Sprague-Dawley rats). Recordings were by whole-cell patch clamp with $KMeSO_4$-containing electrodes. TOLB (0.05-1 mM) depressed afterhyperpolarizations (AHPs) seen after bursts of spikes evoked with depolarizing pulses: the initial peak (medium or mAHP) by 60.3 ± 8.6 % and the slow AHP (sAHP) by 61.3 ± 10.6 % (n=11). The EC_{50} was ≈0.18 mM for the mAHP and 0.34 mM for the sAHP. In 5 cells clamped at ≈ -50 mV, TOLB reduced I_{out} by 65.3 ± 5.3 % and tail currents by 57.2 ± 14.1 %. The TOLB-sensitive I_{out} is Ca-dependent as TOLB (1 mM, 15 min) had no significant effect on I_{out} in Mn^{2+} /low Ca^{2+} medium (-10.5 ± 6.4 %, n=5). TOLB probably acts independently of any changes in Ca-currents because TOLB induced only minor (non-significant) depressions of sustained HVA Ca-currents (by -19.5 ± 9.6 %, n=5). TOLB also depressed M-current (I_M) by 40.9 ± 12.9 % (obtained from inward relaxations evoked by hyperpolarizing pulses from V_H=-30 mV, n=7). Judging by these results, TOLB is not a selective K_{ATP} antagonist in CA1 neurons, since it also depresses voltage- and Ca-dependent I_{out}'s (I_M, $I_{K(Ca)}$). The suppression of several I_{out}'s explains why TOLB enhances excitability and ongoing firing in hippocampal slices.

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720.16

BLOCK BY LOCAL ANESTHETIC OF TRH EFFECTS ON K^+ CURRENTS IN NEUROENDOCRINEL CELLS. Z.-L. XIONG, F. POPITZ-BERGEZ* AND G. STRICHARTZ. ANES. RES. LAB., BWH, HARVARD MED. SCH., BOSTON, MA 02115.

In rat anterior pituitary tumor (GH_3) cells two types of voltage dependent potassium currents (I_K), a slow-inactivating I_s and a fast-inactivating I_{To}, were observed using whole-cell voltage clamp. Under physiological conditions, thyrotropine-releasing hormone (TRH: 0.01 - 1 μM) evoked a dose-dependent, biphasic response of both types of I_K: a transient increase, with peak effect (120 - 360 % of control) at 1 min, followed by a sustained inhibition (60 - 92% of control). This response was repeatable after washout of TRH for 6 min or longer. When a high concentration (4 mM) of EGTA was present intracellularly and 0.2 mM cadmium was added to the bath to block Ca^{2+} entry to the cell, the stimulatory phase from TRH disappeared whereas the inhibitory phase remained unchanged, indicating that the transient stimulatory phase is a Ca^{2+} dependent response (i.e., $I_{K(Ca)}$). On the other hand, when GDP-β-S (0.5 mM) was applied intracellularly, both responses of I_K to TRH were almost abolished, implying a G-protein mediated effect. Lidocaine could inhibit I_K completely (IC_{50} of 1.9 mM) and also inhibit I_{Ca} (IC_{50} of 2.6 mM). In addition, pretreatment (5 - 8 min) of cells with much lower doses of lidocaine (10 - 100 μM, at which I_K was unaffected), largely blocked the effect of TRH. Simultaneous application of lidocaine (100 μM) with TRH (1 μM) failed to block the hormone-induced response. Thus, local anesthetics inhibit the G-protein mediated response in excitable cells at concentrations that do not block ion channels directly. Lidocaine at such concentrations is known to have substantial effect as a systemic analgesic. (Supported by NIGMS 15904).

720.17

CAPSAICIN BLOCKS K^+ AND Ca^{++} CURRENTS AND SELECTIVELY ACTIVATES SENSORY CELLS IN SPINAL NEURONS OF *XENOPUS* EMBRYOS. F. M. Kuenzi* and N. Dale. School of Biological and Medical Sciences, University of St Andrews, KY16 9TS, UK

Besides its selective activation of the slow-conducting pain fibres in mammals, capsaicin also potently blocks voltage-gated ion channels, showing selectivity in some preparations. To test whether this compound could be used to separate fast and slow components of the voltage-gated K^+ currents in *Xenopus*, whole-cell patch-clamp recordings were made from neurons that had been dissociated from spinal cords of stage 37/38 embryos and maintained in culture for up to six hours. In all cells 100 μM capsaicin reduced the potassium current by 88±3.6% (mean ± sem, n=5), 95% maximally, suggesting that both conductance components are affected. The blocking reaction had a K_D = 21 μM and a Hill coefficient of 1.72, suggesting that two molecules of capsaicin may be required. The block was time dependent, having little effect on the time course of activation, but causing an increase in apparent inactivation. The rate of inactivation increased with the concentration of capsaicin. This suggests that capsaicin may act to block the open channel.

Ca^{++} currents were also sensitive to capsaicin, being reduced at 10 μM by 11.4± 1.8% (n=12). Although the voltage-dependence of activation was not affected, capsaicin caused a marked increase in the rate of inactivation of the current in some cells during a 70 ms step.

A proportion of cells that had the appearance of Rohon-Beard sensory neurons in culture (Dale, 1991, *Europ. J Neurosci.* 3, 1025-1035), also responded to low doses of capsaicin with an inward current that was slow to activate and dose-dependent. This response may be analgous to the effects of capsaicin on mammalian C fibres and cultured DRG neurons.

720.18

ZINC MODULATES EXTRACELLULAR FATTY ACID BLOCK OF RECOMBINANT VOLTAGE-GATED POTASSIUM CHANNELS EXPRESSED IN MAMMALIAN CELLS J.S. Poling*[1,3], S. Vicini[1], M.A. Rogawski[2], and N. Salem, Jr.[3] [1]Dept. of Physiology and Biophysics, Georgetown Univ., Wash., DC 20007;[2]NINDS/NIH, Bethesda, MD 20892;[3]NIAAA/NIH, Rockville, M.D. 20852.

Docosahexaenoic acid (DHA), an omega-3 fatty acid, is the major polyunsaturated fatty acid (PUFA) found in mammalian brain and is selectively incorporated into the membrane phospholipid pool of retina and synapses. We have previously reported that certain PUFAs potently blocked native voltage-gated K^+ channels (K_d = 2.5 μM, DHA) and exert their action directly from the extracellular side of the membrane by an open-channel blocking mechanism (Poling et al., Mol. Pharm. 47(2), 1995). In CL1023 fibroblasts stably expressing Kv1.2 or Kv3.1 channels, DHA also blocked whole-cell K^+ currents and accelerated the current decay in a concentration-dependent manner (Kv1.2, K_d = 1.8 μM; Kv3.1, K_d = 690 nM). Block was observed in the whole-cell or outside-out patch configuration indicating that the interaction between DHA and the K^+ channel occurred at an extracellular site. Divalent zinc (10^{-4} M) non-competitively inhibited block of Kv1.2 to a greater extent than Kv3.1. Zinc is present within some neurotransmitter containing vesicles and can be co-released into the synaptic cleft suggesting a novel role for 22:6n3 and zinc as modulators of K^+ channels.

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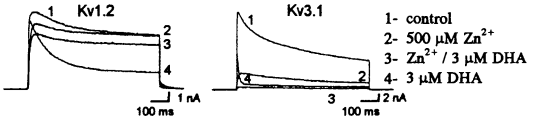

720.19

A ROLE FOR ARACHIDONIC ACID IN GENERAL ANESTHETIC ACTION D. D. Denson and D. C. Eaton*. Depts of Anesthesiology and Physiology and The Center for Cellular and Molecular Signaling, Emory Univ. Sch. of Med., Atlanta, GA 30322.

General anesthetic block of BK channels in GH3 cells is mimicked by PLA_2 inhibitors and attenuated by exogenous arachidonic acid (AA). Untreated GH3 cells continuously produce measurable levels of AA implying tonic PLA_2 activity. PLA_2 activity in GH3 cells can be blocked by concentrations of the intravenous general anesthetic, ketamine, that are comparable to those used clinically (100μM). If general anesthetic block of BK channels is mediated by an inhibition of PLA_2, then addition of exogenous AA should attenuate the anesthetic block and reduce the slope of the ketamine dose response curve. To test this hypothesis, we examined the dose response curve for ketamine in the presence and absence of AA. Single channel recording techniques in excised patches were used to examine the effect of racemic ketamine (10, 50, 100, 500 and 1000μM) on the BK channel activity in GH3 cells. Solutions containing 150 mM KCl were used in both the pipette and bath. Ca_i^{+2} in the bath was buffered to 0.1 μM with 5mM K_2EGTA. AA by itself increased the open probability of BK channels. Ketamine reduced the open probability of channels in the presence and absence of AA, but the ketamine block was significantly different for channels activated by AA (1 μM) compared to channels in absence of AA. Exposure of BK channels to AA caused not only a shift in the ketamine dose response curve to the right as evidenced by a significant ($p<0.001$) increase in EC_{50} from 3.4 ± 0.4 to 693 ± 379 μM but a significant ($p<0.001$) decrease in slope from 1.40 ± 0.21 to 0.59 ± 0.05. These data suggest that exogenous AA is activating BK channels and attenuating general anesthetic block by increasing the membrane concentration of AA that is reduced by general anesthetic inhibition of PLA_2. Since PLA_2 is inhibited by all of the general anesthetics we have examined, disruption of AA production may be an important mechanism for the action of general anesthetics on ion channels within the central nervous system.

720.20

THE POTASSIUM CHANNEL BLOCKERS, 4-AMINOPYRIDINE AND TETRAETHYLAMMONIUM, INCREASE THE SPONTANEOUS BASAL RELEASE OF [3H]5-HYDROXYTRYPTAMINE IN RAT HIPPOCAMPAL SLICES. L.E. Schechter*. Wyeth-Ayerst Research, CNS Disorders, Pearl River, NY 10965.

Previous investigations have demonstrated that compounds capable of blocking presynaptic K+ channels can stimulate neurotransmitter release at both peripheral and central synapses. This study examined the *in vitro* effects of the "classical" K+ channel blockers, 4-aminopyridine (4-AP) and tetraethylammonium (TEA), on the spontaneous basal release of [3H]5-hydroxytryptamine ([3H]5-HT) from rat hippocampal slices using an automated superfusion apparatus. 4-AP and structural analogs increased the spontaneous basal release of [3H]5-HT in a concentration-related manner. The rank order of potencies from the estimated EC50 values indicated that 3,4-DAP(0.88 mM) ≈ 4-AP(1.2 mM) > 2-AP(89 mM) > 3-AP(100 mM) > pyridine(256 mM). TEA stimulated [3H]5-HT release with an estimated EC50 value of 63 mM and was less efficacious than the pyridine congeners. The release induced by 4-AP (0.3, 1 and 10 mM) and TEA (30, 100 and 300 mM) was significantly attenuated in a Ca^{+2}-free buffer containing 1 mM EGTA. Tetrodotoxin (TTX; 1 μM), a Na^+ channel blocker, was unable to block the response of 4-AP (1 mM) and TEA (100 mM). Notably, this concentration of TTX reduced the stimulation of [3H]5-HT release produced by the Na^+ channel opener veratridine (5 μM). The enhancement of release induced by 1 mM 4-AP was additive with 100 mM TEA and 5 μM veratridine but not with 25 or 50 mM KCl. Taken together, the results demonstrate that K+ channel blockade can enhance the spontaneous basal release of [3H]5-HT in rat hippocampal slices. These effects are at least partly dependent on extracellular Ca^{+2} and do not appear to be mediated by modulating Na^+ channel function. In conclusion, this is the first direct demonstration that presynaptic K+ channels can regulate 5-HT neurotransmission.

ACETYLCHOLINE RECEPTOR: NICOTINIC—PHARMACOLOGY

721.1

Functional Pharmacology of Neuronal Nicotinic Acetylcholine Receptors (AChRs) from the Human Cell Line IMR-32
Nelson, M.E., Gerzanich, V., Criswell, L., and Lindstrom, J.M.* Dept. of Neuroscience, Univ. of Pennsylvania Medical School, Philadelphia, PA 19104.

The IMR-32 cell line is a human neural crest derived cell-type that has been shown to express neuronal-type AChRs including α3, α5, α7, β2, and β4 subunits (Neurosci. 32: 759-767; FEBS Lett. 312: 67-70). Here we report the pharmacological characteristics of functional AChRs expressed in these cells using patch-clamp electrophysiology in order to identify the subunit composition of native human AChRs by comparisons to recombinant human AChRs expressed in *Xenopus* oocytes. IMR-32 cells responded with relatively robust currents (averaging about 350 pA of maximal current) when exposed to the nicotinic agonists dimethyl-phenylpiperazinium (DMPP), nicotine, cytisine, acetylcholine (ACh) and tertramethylammonium (TMA). All agonists had similar efficacies and their respective EC_{50} values were approximately 7.6, 22.5, 31.2, 59.0, and 1030 μM. Currents activated by ACh (100 μM) were completely antagonized by d-tubocurarine, mecamylamine, and hexamethonium with IC_{50} values of 0.4, 3.2, and 8.5 μM, respectively, while methyllycaconitine (up to 1 μM) was virtually ineffective. Single-channel currents activated in outside-out patches from IMR-32 cells had a chord conductance of 46 pS and distributions of channel open times that were best fit by double exponential functions with time constants of 1.7 and 6.0 ms. Comparisons between IMR-32 nicotinic responses and human neuronal AChRs functionally expressed in *Xenopus* oocytes suggest that the predominant AChR expressed in IMR-32 cells is one including α3 and β4 receptor subunits, although participation of other receptor subunits cannot be excluded based on the present data. (Supported by grants from NINDS, STRC, CTR, and MDA.)

721.2

GALANTHAMINE IS A POSITIVE MODULATOR OF THE α-BUNGAROTOXIN-SENSITIVE HIPPOCAMPAL NICOTINIC RECEPTOR ACTIVITY. E.X. Albuquerque[1,2*], E.F.R. Pereira[1], A. Schrattenholz[3], A. Maelicke[3] [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, USA; [2] Lab. Mol. Pharmacol., Inst. Bioph. "Carlos Chagas Filho", UFRJ, Rio de Janeiro, RJ 21944, Brazil; [3] Inst. Physiol. Chem. Pathobiochem., Johannes-Gutenberg Univ. Med. Sch., Mainz D-6500, Germany.

A new binding site has been identified on neuronal and muscle nicotinic receptors (nAChRs) through which the ion-channel can be activated (*J. Pharmacol. Exp. Ther.* 265: 1474, 1993). This site is referred to as the physostigmine (PHY) site, is insensitive to classical nicotinic agonists such as ACh and anatoxin-a (AnTX), and recognizes as agonists the anticholinesterases PHY and galanthamine (GAL). To determine whether the nAChR activity induced by classical nicotinic agonists can be modulated via this novel site, we used the whole-cell mode of the patch-clamp technique to investigate the effects of GAL on α-bungarotoxin (α-BGT)-sensitive currents elicited by 500-ms pulses of AnTX to cultured hippocampal neurons. When applied to the neurons as an admixture with AnTX (10 μM), GAL (0.1-100 μM) changed neither the peak amplitude nor the decay time constant of AnTX-induced currents. However, following the recording of the AnTX-induced current, perfusion of the neurons for 5 min with GAL (1 μM)-containing external solution prior to their subsequent exposure to the admixture of GAL (1 μM) and AnTX (10 μM) caused an increase of the peak amplitude and a prolongation of the fast decay phase of the AnTX-induced current; the peak amplitude was increased by about 30 % and the decay time constant was prolonged from 23.6 ± 1.9 ms to 30.2 ± 2.1 ms (mean ± SEM, n = 4). These results suggest that a ligand capable of binding to the PHY site may play an important role in regulating the desensitization of the α-BGT-sensitive, presumably an α7-bearing, hippocampal nAChR. (USPHS Grant NS25296).

721.3

α-CONOTOXIN-ImI: A POTENT ANTAGONIST AT THE α-BUNGAROTOXIN (α-BGT)-SENSITIVE HIPPOCAMPAL NICOTINIC RECEPTOR (nAChR). E.F.R. Pereira [1*], M. Alkondon[1], J.M. McIntosh [2] and E.X. Albuquerque [1,3]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, USA; [2]Depts. of Psychiatry and Biology, Univ. Utah, Salt Lake City, Utah 84112, USA; [3]Lab. Mol. Pharmacol., IBCCF, UFRJ, RJ 21944, Brazil.

α-Conotoxin-ImI (α-CTX-ImI), a neurotoxin isolated from the venom of the snail *Conus imperialis*, has been characterized as a disulfide-rich dodecapeptide that (i) acts as a nicotinic antagonist at the frog neuromuscular junction, (ii) does not cause paralysis when injected intraperitoneally in mice, and (iii) causes seizures when injected into the intracerebroventricular space of rats or mice (*J. Biol. Chem.* 269: 16733, 1994). Whereas α-CTX-ImI seems to be a poor antagonist at mammalian muscle nAChRs, it may act as a potent antagonist at mammalian neuronal nAChRs. The fact that α-BGT also causes seizures when injected intracranially in rats and mice led us to test whether α-CTX-ImI interacts with the α-BGT-sensitive, presumably α7-bearing, hippocampal nAChR. Nicotinic whole-cell currents elicited by 500-ms pulses of ACh (1 mM) were recorded from cultured hippocampal neurons using the patch-clamp technique. In this study, α-BGT-sensitive currents (referred to as type IA currents) were characterized by their fast decay and by their reversible inhibition by methyllycaconitine (1 nM) (*J. Pharmacol. Exp. Ther.* 265:1455, 1993). The effect of α-CTX-ImI on type IA currents was evaluated after superfusing the neurons with α-CTX-ImI-containing external solution for 5 min. At 100 nM, α-CTX-ImI decreased the peak current amplitude to 45 ± 2.5% of control (mean ± SEM, n =3). The relationship between the normalized peak current amplitude and the concentration of α-CTX-ImI revealed that the IC_{50} for this toxin in inhibiting type IA current is about 95 nM. The α-CTX-ImI-induced inhibition of type IA currents was reversed by washing. Our results indicate that α-CTX-ImI potently and reversibly inhibits the activation of α-BGT-sensitive hippocampal nAChRs. (USPHS Grants NS25296, ES05730).

721.4

POTENCIES OF MUSCLE RELAXANTS PANCURONIUM AND (+)-TUBOCURARINE AT MUSCLE NICOTINIC RECEPTORS (nAChR). C.M. Garland, L. Holden-Dye, J. Chad, S.D. Jane, R.C. Foreman, R.J. Walker*. Dept. of Physiol. & Pharmacol., University of Southampton, Southampton SO16 7PX, UK.

Clinically, pancuronium is more effective than (+)-tubocurarine (tc) (Kreig *et al.*, 1980, Br. J. Anaesth. 52, 783-787) as a muscle relaxant. We are investigating the molecular basis for their action at nAChRs.

In oocytes expressing nAChRs (mouse muscle, αβγδ) pancuronium (IC_{50} 5.0 ± 1.9 pM; n=4) was more potent at blocking currents induced by 1 μM ACh than tc (IC_{50} 7.4 ± 0.8 nM; n=4). In the rat phrenic nerve diaphragm pancuronium and tc were less potent (IC_{50} 2.5 μM, n=2 and 5.0 μM, n=2, respectively). The possibility that the potency of pancuronium is partly determined by the expression system/tissue is being investigated by comparison with effects on mouse muscle myoblasts (which also express αβγδ subunits). Confocal microscopy of Indo-1-AM loaded myoblasts is used to detect increases in intracellular Ca^{++} activity ($aCa^{++}i$) an indirect measure of nAChR activation. 10 μM ACh increased $Ca^{++}i$ from 100-200nM to 400-600nM and this was blocked by 1 μM pancuronium. We are currently quantifying the potency of tc and pancuronium in this system.

cDNA clones were kindly donated by Professor S. Heinemann, Salk Institute, California. CMG is supported by Organon Teknica, Belgium. We thank Dr R.J Marshall and Dr A. Muir for helpful discussions.

721.5

A NOVEL NON-COMPETITIVE *CONUS* PEPTIDE INHIBITOR OF THE NICOTINIC ACETYLCHOLINE RECEPTOR. M. M. Grilley*, K.-J. Shon, C. Hopkins, D. Yoshikami, and B. M. Olivera. Department of Biology, University of Utah, Salt Lake City, UT 84112.

Venomous snails of the genus *Conus* produce a variety of peptide toxins which individually target a range of receptors and ion channels. Venom milked from the fish hunting *C. purpurascens* has been fractionated and found to contain, among others, a peptide unique both structurally and mechanistically among previously characterized conotoxins. The 24 amino acid peptide was chemically synthesized and, like the native material, caused paralysis and death following injection into goldfish. Binding and photoaffinity labeling of the peptide to *Torpedo* electric organ membrane indicate it targets the nicotinic acetylcholine receptor, but that it does not compete with α-bungarotoxin or the α-conotoxins, competitive inhibitors of α-bungarotoxin binding on the muscle subtype of the acetylcholine receptor. Electrophysiology studies suggest the peptide acts as a non-competitive inhibitor of the acetylcholine receptor by physically blocking the pore. Further analysis of analogs of this peptide will allow determination of functional groups important for the peptide-channel interaction. (Supported by NIDA grant #DA05485.)

721.6

INHIBITORY EFFECTS OF THE ANTIVIRAL AGENT AMANTADINE ON NICOTINIC ACETYLCHOLINE RESPONSES IN RAT HIPPOCAMPAL CULTURED NEURONS. H. Matsubayashi* and E.X. Albuquerque. Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, USA.

Amantadine is used for the treatment of influenza and Parkinson's disease. On frog sartorius muscle, this drug decreases the amplitude of the endplate current and reverses the slope of the relation between half-decay time and membrane potential, suggesting an alteration of the nicotinic acetylcholine receptor/ion channel (nAChR) (*Science* 199:788, 1978). In the present study the effects of amantadine on hippocampal nAChRs were investigated. At least three distinct current types, IA, II and III, have been recorded from hippocampal neurons (*J. Pharmacol. Exp. Ther.*, 265:1455, 1993), and they are subserved by nAChRs with different subunit compositions. In this study, whole-cell currents were recorded from cultured hippocampal neurons according to standard patch-clamp technique. The external perfusing solution contained atropine (1 μM) and tetrodotoxin (300 nM). The results showed that amantadine (500 μM) decreased the peak amplitude of all three types of currents elicited by pulse application (0.5-2 s) of ACh (1 mM) to the cultured neurons. When amantadine was simultaneously applied via the background perfusion and as an admixture with ACh via the U-tube, and ATP-regenerating solution was used to prevent rundown of type IA current, the IC_{50} for amantadine in inhibiting ACh (0.3 mM)-induced type IA current was 6.21 μM. Amantadine was more potent in inhibiting the activation of nicotinic currents in hippocampal neurons than in inhibiting endplate currents (*Mol. Pharmacol.* 22:82, 1982). It is concluded that amantadine is a more potent antagonist of neuronal nAChRs than of muscle nAChRs. (*Support*: USPHS Grants NS25296 and ES05730)

721.7

DEVELOPMENT OF A NOVEL CLASS OF NICOTINIC RECEPTOR ANTAGONISTS: INHIBITION OF NICOTINE-EVOKED STRIATAL DOPAMINE RELEASE AND THE ELECTROPHYSIOLOGICAL RESPONSE OF A CLONED NICOTINIC RECEPTOR SUBTYPE IN *XENOPUS* OOCYTES. P.A. Crooks, L.H. Teng, A. Ravard, S.T. Buxton, B.N. Cohen, D.D. Allen* and L.P. Dwoskin. College of Pharmacy, Univ. of Kentucky, Lexington, KY 40536 and Div. of Biomedical Sciences, Univ. of California, Riverside, CA 92521.

We have undertaken the development of subtype selective antagonists of the neuronal nicotinic receptor site. A series of pyridyl N-substituted nicotine analogues were synthesized and evaluated for their ability to inhibit nicotine (10 μM)-evoked [^{3}H]dopamine ([^{3}H]DA) release from rat striatal slices. Antagonist potency increased with increasing carbon number. The N-octyl analogue NONI was the most efficacious and potent in the series, and afforded greater inhibitory efficacy than the competitive neuronal nicotinic receptor antagonist, dihydro-β-erythroidine (DHβE) and the nicotinic receptor channel blocker, mecamylamine (MEC). The N-allyl analogue NANI was less potent than NONI and had similar efficacy to DHβE. The order of potency in the release assay was NONI>DHβE>MEC>NANI, with IC50s of 1.3, 2.5, 3.0, 12 μM, respectively. In electrophysiological studies, NONI blocked the response of cloned α3β2 nicotinic receptors to 1 μM acetylcholine by 50% at 5 μM, and by 100% at 100 μM, and displayed no agonist activity. These data suggest that the antagonists may block the α3β2 receptor to inhibit nicotine-evoked [^{3}H]DA release from striatum. Molecular modeling data suggest that this new class of antagonist binds in a novel manner to the nicotinic receptor. (Supported by a grant from the Tobacco and Health Research Institute, Lexington, KY).

721.8

DEVELOPMENT OF A NOVEL CLASS OF NICOTINIC RECEPTOR ANTAGONISTS: DISPLACEMENT OF [^{3}H]NICOTINE BINDING FROM RAT STRIATAL MEMBRANES. L.H. Wilkins, P.A. Crooks, A. Ravard, R.A. Yokel* and L.P. Dwoskin. College of Pharmacy, University of Kentucky, Lexington, KY 40536.

To study the mechanism of action of nicotine in the CNS, we have undertaken the development of subtype selective antagonists of the neuronal nicotinic receptor site. A series of N-substituted nicotine analogues were synthesized and evaluated for their ability to displace [^{3}H]nicotine binding from rat striatal membranes. The effects of the analogues were compared to the competitive nicotinic receptor antagonist dihydro-β-erythroidine (DHβE). Scatchard analysis of [^{3}H]nicotine binding revealed a Kd = 1.46 ± 0.15 nM and Bmax = 117 ± 9.05 fmol/mg protein. In displacement studies, the Ki's for nicotine, DHβE, S(-)N-allylnicotinium iodide (NANI), S(-)N-methylnicotinium iodide (NMNI) and S(-)N-octylnicotinium iodide (NONI) were 0.38 ± 0.11 nM, 64.9 ± 10.4 nM, 2.38 ± 0.24 μM, 3.10 ± 0.40 μM and 20.1 ± 1.6 μM, respectively. Moreover, the order of potency for displacement of [^{3}H]nicotine binding does not correlate with the order of potency for inhibition of [^{3}H]dopamine release from rat striatal slices (Crooks et al, Soc. Neurosci., 1995). This poor correlation suggests that the receptor subtypes that control [^{3}H]dopamine release differ from the subtypes that are primarily responsible for high-affinity [^{3}H]nicotine binding. Thus, the analogues had marginal affinity for the [^{3}H]nicotine binding site in rat striatum compared with DHβE, suggesting that they do not have a significant affinity for the α4β2 neuronal nicotinic receptor subtype, but may be selective for the α3β2 subtype. (Supported by a grant from the Tobacco and Health Research Institute, Lexington, KY).

721.9

PHARMACOLOGICAL COMPARISON OF NICOTINE-EVOKED RELEASE OF STRIATAL DOPAMINE AND HIPPOCAMPAL NORADRENALINE FROM SUPERFUSED SYNAPTOSOMES
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In situ hybridization studies suggest that rat nigrostriatal dopamine neurons express mainly α4, α5, and ß2 nicotinic receptor subunits, whereas dorsal bundle noradrenaline neurons express mainly α3, ß2 and ß4 subunits (Wada et al 1989, 1990; Dineley-Miller and Patrick 1992). In order to test for pharmacological differences, agonist-evoked release of striatal ^{3}H-DA and hippocampal ^{3}H-NA was measured from superfused synaptosomes prepared from rat. Nicotine, cytisine, DMPP and ACh (with esterase inhibitor and muscarinic antagonist) increased NA release in a concentration-dependent manner (EC50s of 3.7 µM, 6.0 µM, 8.9 µM and 13 µM, respectively). Maximal drug effects were similar for each agonist. DA was released more potently than NA by L-nicotine (EC50 0.16 µM vs. 6.4 µM) and anatoxin (EC50 0.035 µM vs 0.39 µM). Isoarecolone (1 – 320 µM) released DA more than NA at each concentration, but a maximal effect was not reached. Lobeline evoked DA release but not NA release. High K^+ (10 mM) released DA and NA to similar extents. The nicotinic antagonist mecamylamine (10 µM) virtually abolished NA and DA release evoked by high concentrations of all agonists tested except lobeline. The nicotinic antagonists DHBE and methyllycaconitine inhibited nicotine-evoked DA release more potently than NA release, each with a 45-fold DA:NA potency difference when tested against EC50 values for nicotine. Thus, nicotinic receptors associated with striatal DA and hippocampal NA afferents have different pharmacological profiles that may be attributable to their subunit composition.

721.10

EFFECTS OF A-85380 ON NICOTINIC RECEPTOR MEDIATED $^{86}Rb^+$ EFFLUX FROM MOUSE THALAMIC SYNAPTOSOMES.
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A-85380 [3-(2(S)-azetidinylmethoxy)pyridine], a novel, potent nicotinic agonist that activates a variety of human nicotinic receptor subtypes, was evaluated for its ability to stimulate the efflux of $^{86}Rb^+$ from synaptosomes prepared from thalamus of C57BL/6 mice. The stimulation of $^{86}Rb^+$ efflux by A-85380 was concentration dependent, but apparently biphasic. One component had a very high affinity (EC_{50} = 4.2 nM) and a maximal response approximately equal to that measured for 10 µM nicotine. A second component, that did not saturate by 3 µM, was observed at concentrations above 300 nM. The $^{86}Rb^+$ efflux measured using either 30 nM or 3 µM A-85380 were each inhibited approximately 90% by 10 µM mecamylamine. Exposure to stimulating concentrations (0.3-10 nM) of A-85380 desensitized $^{86}Rb^+$ efflux. An EC_{50} value about 4 nM was calculated for both stimulation and desensitization of efflux. Desensitization of the efflux stimulated by 10 µM nicotine was also observed with concentrations of A-85380 that elicited little efflux (IC_{50} = 0.2 nM). The desensitization rate for 0.3 nM A-85380 (0.27 min^{-1}) was lower than the maximum rate observed for activating concentrations (2.4 min^{-1}). Synaptosomes that had been desensitized by exposure to either nonstimulating (0.3 nM) or stimulating (30 nM) concentrations of A-85380 partially recovered responsiveness after the termination of treatment (75% and 35%, respectively). The recovery from desensitization after treatment with 0.3 nM was faster ($k=0.34^{-1}$) than that after 30 nM ($k=0.19$ min^{-1}). The kinetics of desensitization observed after exposure to either stimulating or nonstimulating concentrations of A-85380 were comparable to those measured previously for the extremely potent nicotinic agonist, epibatidine. These studies indicate that A-85380 is among the most potent agonists yet tested at nicotinic receptors in the CNS. Supported by Abbott Laboratories.

721.11

CHOLINERGIC ACTIVATION OF THE ELECTROCORTICOGRAM: AN AMYGDALAR ACTIVATING SYSTEM.
H.C.Dringenberg, and C.H.Vanderwolf*. Neuroscience Program, University of Western Ontario, London, Ontario, Canada, N6G 2V4.

In urethane-anesthetized rats, 100 Hz electrical stimulation of the basal amygdala changed the slow wave activity of the neocortex from 2-6 Hz large irregular slow activity (LISA) to low voltage fast activity (LVFA) including frequencies of above 10 Hz. A similar activating effect was seen in the hippocampus where amygdala stimulation induced the appearance of rhythmical slow activity (RSA) in the 2-6 Hz range. This activation of neocortical and hippocampal slow wave activity by amygdala stimulation was blocked by the cholinergic-muscarinic receptor antagonist scopolamine (0.5-5.0 mg/kg, i.p.), but not by the peripheral antagonist methyl-scopolamine, in a concentration-dependent manner. In contrast, a blockade of ascending inputs from the midbrain to the neocortex by treatment with the serotonin-depletor *p*-chlorophenylalanine or cauterization of the rostral midbrain did not block neocortical LVFA to amygdala stimulation, even though the lesions abolished all LVFA to strong noxious stimuli such as tail pinches. Unilateral infusions of the local anesthetic lidocaine (1%) into the basal forebrain selectively blocked LVFA in the neocortex ipsilateral to the infusion. However, intracerebral or systemic administration of various excitatory amino acid antagonists (2-amino-5 - phosphonovaleric acid, kynurenic acid, NPC 12626) was not effective in blocking LVFA to amygdala stimulation. Extracellular single unit recordings in the basal forebrain showed that about 50% of cells that were more active during periods of neocortical LVFA relative to LISA were excited by single pulse stimulation of the basal amygdala. Cells that were more active during LISA often were inhibited by amygdala stimulation. The basal amygdala appears to provide an important excitatory input to the basal forebrain cholinergic system involved in activation of the electrocorticogram. (Supported by NSERC, Canada).

EXCITATORY AMINO ACID RECEPTORS: RECEPTOR LOCALIZATION

722.1

SELECTIVE VULNERABILITY OF NEURONS IN THE HUMAN NUCLEUS BASALIS OF MEYNERT: PUTATIVE ROLE OF AN AGE-RELATED DECREASE IN GLUR2/3 RECEPTOR SUBUNIT IMMUNOREACTIVITY.
M.D. Ikonomovic*, R. Sheffield, and D.M. Armstrong. Allegheny-Singer Research Institute, Medical College of Pennsylvania, Pittsburgh, PA 15212.

Polyclonal antibodies directed against the GluR1 and GluR2/3 subunits of the AMPA receptor complex were employed in order to examine in brains of elderly humans (average age = 75) the distribution and subunit composition of AMPA-selective receptors within magnocellular neurons comprising the nucleus basalis of Meynert (NBM). Within each case (n = 9) NBM neurons were intensely labeled for GluR1, yet displayed virtually no immunolabeling for GluR2/3. In contrast, GluR2/3, but not GluR1, labeled neurons were abundantly observed within adjacent brain regions of these same cases (*i.e.*, amygdala, hippocampus and entorhinal cortex). Collectively, these data suggest that within elderly subjects the molecular composition of AMPA receptors may differ between neurons in the NBM and surrounding regions. In addition, we examined three younger cases (ages 5, 33 and 50 y) in order to assess whether the paucity of GluR2/3 immunolabeling within the NBM of elderly subjects was a consequence of normal aging or consistent throughout life. Within the 5 and 33 year old cases we observed GluR2/3-labeled neurons within the NBM, although the intensity of immunolabeling was relatively modest within the younger case. In contrast, little if any GluR2/3-labeling was observed on NBM neurons in the 50 year old cases, although robustly labeled neurons were present within adjacent brain regions. Although preliminary these latter data suggest that with advancing age there is a selective decrease in GluR2/3 immunoreactivity within NBM neurons, we hypothesize that the loss of the GluR2/3 subunit may render these neurons functionally distinct with respect to their responsiveness to glutamate. Therefore, alterations in the molecular composition of the AMPA receptor may contribute to the selective vulnerability of these cells to excitotoxic insults and to their severe loss in a number of neurodegenerative diseases including Alzheimer's disease.

722.2

AMPA-SELECTIVE GLUTAMATE RECEPTOR SUBTYPE IMMUNOREACTIVITY IN THE HIPPOCAMPAL FORMATION OF PATIENTS WITH ALZHEIMER'S DISEASE: EVIDENCE FOR HIPPOCAMPAL PLASTICITY.
D.M. Armstrong*, R. Sheffield, and M.D. Ikonomovic. Allegheny-Singer Research Institute, Medical College of Pennsylvania and Hahnemann University, Allgheny Campus, Pittsburgh, PA 15212.

Immunocytochemical techniques were employed in order to examine the distribution and intensity of immunolabeling for AMPA receptor subunits GluR1 and GluR2/3 within the hippocampal formation of normal controls and Alzheimer's disease (AD) cases. Throughout our investigation we examined cases exhibiting a wide range of pathologic severity, thus allowing us to correlate our immunohistochemical data with extent of pathology. Specifically, we investigated the distribution of these receptor subunits in hippocampal sectors which are particularly vulnerable to AD pathology (*i.e.*, CA1 and subiculum) and compared these findings to those obtained following examination of sectors which are generally resistant to pathologic change (*i.e.*, CA2/3, dentate gyrus). Within vulnerable sectors we observed a variable loss of GluR1 and GluR2/3 immunolabeling. The degree to which these proteins were reduced correlated with the extent of neurofibrillary pathology and cell loss. Despite the loss of labeled cells the intensity of immunolabeling within remaining neurons was comparable to and in many instances even greater than that observed in control cases. Within resistant sectors, the distribution of immunoreactive elements was comparable in both case groups yet the intensity of immunolabeling was markedly increased in AD cases, particularly in the molecular layer of the dentate gyrus and in the stratum lucidum of CA3 (*i.e.*, termination zones of perforant pathway and mossy fibers). In addition, within AD cases dramatic increases were observed within the surpagranular and polymorphic layer of the dentate gyrus (*i.e.*, terminal zones of sprouting mossy fiber collaterals). The increase in GluR1 and GluR2/3 immunolabeling is hypothesized to occur in response to the deafferentation of selected glutamatergic pathways. Moreover, our data support the concept that hippocampal plasticity is preserved, even in severe AD cases, and suggest a critical role for AMPA receptor subunits in maintaining hippocampal functioning.

722.3

AMPA-SELECTIVE GLUTAMATE RECEPTOR SUBUNITS IN THE VISUAL AND PARIETAL CORTICES OF NORMAL CONTROLS AND PATIENTS WITH ALZHEIMER'S DISEASE. A. Oguntola, R. Sheffield*, M.D. Ikonomovic, and D.M. Armstrong. Medical College of Pennsylvania and Hahnemann University, Allegheny-Singer Research Institute, Pittsburgh PA 15212.

We employed immunocytochemical techniques and antibodies against the AMPA-receptor subunits GluR2/3 to examine the distribution of these receptor subunits within the visual (Areas 17 and 18) and parietal cortices of normal elderly subjects and patients with Alzheimer's disease (AD). These studies are part of a continuing effort to investigate the selective vulnerability of glutamatergic neurons in AD. These regions were selected for study because they represent areas of brain that express abundant (*i.e.*, parietal cortex) and modest (*i.e.*, visual cortex) AD neuropathology. Within the visual cortex, we observed GluR2/3-labeled pyramidal and non-pyramidal neurons, with the latter being the more predominant. In area 17, the majority of GluR2/3-positive neurons were distributed within layers V and VI. In contrast, within area 18 both cell types were observed within layers V and VI as well as within layers II and III. When these same regions were examined in AD cases no significant reduction in GluR2/3 labeled neurons was observed. Within the parietal cortex, GluR2/3 pyramidal and non-pyramidal neurons were observed, with pyramidal neurons being the more abundant cell type. As in Area 18, these cells were distributed throughout layers II and III as well as within layers V and VI. Labeled non-pyramidal neurons were not reduced in the parietal cortex in AD compared to controls. In contrast, GluR2/3-positive pyramidal neurons were reduced by 70% within layers II and III and 62% within layers V and VI of the parietal cortex of AD cases compared to controls. These latter data suggest that in the parietal cortex GluR2/3 pyramidal neurons are particularly vulnerable to pathologic insult. We are now examining the extent to which AMPA receptors contribute to the degeneration of these cells.

722.4

CHARACTERISATION AND LOCALISATION OF ALTERNATIVE SPLICED ISOFORMS OF THE NMDAR1 mRNA IN THE HUMAN HIPPOCAMPUS. X.F. Huang*, G. Dixon, S.V. Catts, P.B. Ward, N. Di Girolamo, D. Wakefield, G. Paxinos, and A. Lloyd. Schools of Pathology, Psychiatry, & Psychology. UNSW, Sydney 2052, Australia

The gene for human N-methyl-D-aspartate receptor 1 (NMDAR1) has recently been cloned and in the rat eight alternatively spliced, regionally distributed, and functionally unique isoforms have been identified. We sought to define the presence and distribution of alternatively spliced NMDAR1 mRNAs in adult human hippocampus. Human brains were obtained at autopsy from five individuals without neurological disease with postmortem delay of 4 to 24 hours. Total RNA was prepared from specific regions of human hippocampal tissue. Reverse transcriptase polymerase chain reactions was used to amplify across the two predicted splice junctions of the human NMDAR1 cDNA to identify possible isoforms in the human gene. The amplified cDNAs were characterised by Southern hybridisation using oligonucleotide splice junction probes specific for each of the unique splice sequences. In the amino terminal region, three apparent splice isoforms amplified from the rat brain RNA and four amplified products were seen in the human hippocampal RNA. In the carboxyl terminal region, there were five amplified products in both rats and human. Northern analysis with rat brain and human hippocampal RNA demonstrates a band at 4.4 Kb prolonged gel electrophoresis and in Northern hybridisation it allowed preliminary definition of regional quantitative differences in the NMDAR1 mRNA isoforms. The in situ hybridisation studies showed an heterogenous distribution of NMDAR1 isoforms in the human hippocampal formation. The dentate gyrus, CA4, and CA3 had a similar intensity of mRNA expression of all six splice isoforms, whereas NMDAR1-2 isoform appeared to have considerably lower expression in CA1 and was almost absent in the parasubiculum. These data may provide clues to the differential susceptibility to pathological processes including ischaemia, epilepsy, and schizophrenia. Supported by the NH&MRC of Australia.

722.5

IMMUNOLOCALIZATION OF GLUTAMATE AND GLUTAMATE RECEPTORS IN THALAMOCORTICAL, THALAMOSTRIATAL AND CORTICOSTRIATAL SYSTEMS IN TURTLES. M. Fowler* and A. Reiner. Dept. Anatomy & Neurobiology, University of Tennessee, Memphis, TN 38163.

Although thalamocortical, thalamostriatal and corticostriatal projection systems are present in turtles, it is uncertain if these systems utilize glutamate as a neurotransmitter, as has been shown to be the case in mammals. Immunohistochemical localization of glutamate and three of the AMPA type glutamate receptors subunits (GluR2/3 and GluR4) were used to address this issue. Glutamate immunolabeling was found to be intense in essentially all neurons of the dorsomedial and dorsolateral thalamic nuclei in turtle, which appear to correspond to the mammalian intralaminar thalamic nuclei. Intense glutamate immunolabeling was also observed in neurons of the nucleus reuniens, and less intense glutamate labeling was observed in neurons of the nucleus rotundus and dorsal lateral geniculate nucleus. These neurons relay auditory (reuniens), visual tectal (rotundus) and retinal (geniculate) information to the telencephalon. Within the telencephalon, the pallial cell groups receiving thalamic input (i.e. the dorsal cortex, pallial thickening and the dorsal ventricular ridge) were in rich in glutamatergic neurons. Additionally, these regions were pervasively rich in neurons possessing GluR2/3 subunits and a small subpopulation were rich in GluR4 subunits. A similar distribution of GluR2/3 and GluR4 subunits was found in the striatal part of the basal ganglia, and the pallidal region was specifically rich in large neurons possessing GluR4 subunits. These results indicate the thalamostriatal, thalamocortical and corticostriatal projections systems of turtles to be glutamatergic. Supported by NS-19620, EY-05298 (A.R.).

722.6

HUMAN NT2-N NEURONS EXPRESS THE NR1 and NR2B SUBUNITS OF THE NMDA RECEPTOR **P. McGonigle*, L. Lu, B.B. Wolfe and M. Munir Departments of Pharmacology, University of Pennsylvania and Georgetown University.**

In this study, we used modulation of glutamate excitotoxicity to characterize pharmacological properties and specific antibodies to detect individual subunits of NMDA receptors expressed by terminally differentiated NT2 neurons. The glycine site antagonist 7-chlorokynurenic acid completely blocked glutamate toxicity in a dose-dependent manner. The polyamine agonists spermine and spermidine enhanced glutamate toxicity in a dose-dependent manner consistent with expression of a NR1-NR2B combination of subunits. The polyamine antagonists putrescine and di-ethylene triamine had no effect on toxicity. Surprisingly, the putative inverse agonists diaminodecane and diaminododecane also enhanced toxicity in a dose-dependent manner. The atypical antagonist ifenprodil completely blocked glutamate toxicity with a uniformly high affinity characteristic of interaction with the NR1-NR2B combination of subunits. Approximately 300 fmol/mg protein of the NR1 subunit was detected with a specific antibody directed against a fusion protein containing a region common to all of the known splice variants of the NR1 subunit. Approximately 700 fmol/mg protein of the NR2B receptor was detected with a specific antibody directed against this subunit. Neither protein was detectable in undifferentiated cells. In contrast, only 12 fmol/mg of the NR2A subunit was detected in NT2-N cells and 7 fmol/mg protein was detected in undifferentiated cells. The pharmacological and immunological results indicate that a functional NR1-NR2B combination of subunits are expressed by these cells. There is no pharmacological evidence that these cells form a functional combination of NR1 and NR2A subunits. The effects of the inverse agonists may indicate the expression of a novel splice variant of the NR1 subunit in these cells. (Supported by NS-08803 and GM-34781)

722.7

N-METHYL-D-ASPARTATE RECEPTOR (NMDAR1) SPLICE VARIANTS DIFFER IN SELECTED REGIONS OF RAT CNS. M. Brodsky*, K. Elliott, B. Warwick and C. E. Inturrisi. Department of Pharmacology, Cornell University Medical College, New York, NY 10021.

The NMDA receptor plays an important role in excitatory neurotransmission and neuronal plastisity, and is thought to be involved in the mechanisms of chronic pain and in mu opioid tolerance. Pharmacologically distinct (homomeric) receptors are expressed from the eight cloned splice variants of the NMDAR1 subunit (1a,1b through 4a,4b) (Hollmann et al., Neuron 10:943, 1993). Thus, the pharmacological profile of a neuronal population may depend on the ratio of splice variants it expresses. We have used a ribonuclease protection assay followed by phosphorimager analysis to quantitatively define the NMDAR1 splice variant ratios for selected regions of rat CNS involved in nociceptive processing and opioid antinociception, and for the cerebellum (Cblm). ^{32}P-labelled riboprobes were directed against the N- and C-terminal splice sites, and tailored (by PCR) to yield different size protected fragments. The ratio of the -a to -b types of NMDAR1 splice variants is 1:2 in the spinal cord dorsal horn (SpC), 3:2 in nucleus raphe magnus (NRM), 1:1 in medial hypothalamus (Hyp), 5:1 in periaqueductal grey (PAG), 2:1 in midline thalamus (Thal), 5:2 in sensorimotor cortex (Ctx), and 1:1 in Cblm. The ratio of the NMDAR1 variants at the C-terminal splice site, i.e. -1 to -2 to -3 to -4, is (in %) 7:23:4:66 in SpC, 5:31:2:62 in NRM, 7:22:5:66 in PAG, 17:18:9:56 in Hyp, 15:26:8:51 in Thal, 41:17:15:27 in Ctx, and 13:31:5:51 in Cblm. Thus, while SpC, NRM and PAG appear to have similar % ratios of the splice variants at the C-terminal site, they differ markedly from the ratios in diencephalon, Ctx, and Cblm. Our results illustrate the differential distribution of the NMDAR1 splice variants in rat CNS, which may contribute to the regional variation in the pharmacological properties of the NMDA receptor. Supported by NIDA Grants DA07274, DA01457, DA00198, and the VZV Research Foundation.

722.8

***IN SITU* HYBRIDIZATION STUDIES OF THE DISTRIBUTION OF A CPP-BINDING PROTEIN IN RAT BRAIN.** R. Pal*, K.T. Eggeman, K.N. Kumar, A.E.Allen and E.K. Michaelis. Dept. of Pharmacol. & Toxicol., the Higuchi Biosciences Center, and the Mental Retardation Research Center, Univ. of Kansas, Lawrence, KS 66045.

A complex of proteins that has recognition sites for NMDA receptor ligands was isolated from rat brain synaptic membranes (Kumar et. al., *J. Biol. Chem.* **269,** 27384-27393, 1994). Antibodies to three of the proteins, a 70 kDa glutamate binding protein (GBP), a 54 kDa CPP-binding protein, and a 60 kDa glycine/TCP-binding protein were used to demonstrate the presence of these three proteins in the complex purified from synaptic membranes. The antibodies raised against the CPP-binding protein labeled an 80-83 kDa protein in synaptic membranes and were used to screen hippocampal cDNA libraries and to clone a 3.8 kb cDNA insert (Kumar et al., this meeting). A 1.4 kb PstI fragment of the original 3.8 kb clone was used to synthesize the antisense and sense cRNA probes used in the *in situ* hybridization studies. The probes were labeled with [^{35}S]CTP. The results of the *in situ* hybridization studies were indicative of high levels of expression of the mRNA for the CPP-binding protein in cerebellum, hippocampus, dentate gyrus, and olfactory tubercle. Intermediate levels of expression were detected in cerebral cortex, and relatively low levels in the inferior and superior colliculus. An almost identical pattern of labeling was observed when the antisense cRNA probes for the glutamate-binding protein were used. The anti-CPP-binding-protein antibodies labeled the same population of cells as that labeled by the cRNA probes structured on the basis of the 3.8 kb cDNA that was cloned. Based on the results of both the *in situ* hybridization studies and the immunohistochemical labeling it appeared that the CPP-binding protein of an NMDA receptor-like complex was widely distributed in the rat brain and was most highly expressed in areas of strong glutamatergic activity. (Supported by grants AA04732 and HD90-07).

722.9

MEMANTINE INDUCES HEAT SHOCK PROTEIN HSP-70 IN THE POSTERIOR CINGULATE AND RETROSPLENIAL CORTEX OF RAT BRAIN. S.Tomitaka*, K.Hashimoto, N.Narita, Y.Minabe and A.Tamura. Natl. Inst. Neurosci., NCNP, Tokyo and Dept. of Psychiatry, Tokyo Women's Medical College

NMDA antagonists, e.g. MK-801 have been investigated for their therapeutic potential in brain ischemia and other neuropathological disease. However, the clinical potential of these agents may be limited by the observations that these NMDA antagonists cause neuronal injury or reversible neuronal swelling. This damage is manifest as a vacuolization in neurons of the cingulate and retrosplenial cortices as well as induction of the 70 kDa heat shock protein (HSP-70) in neurons. Memantine (1-amino-3,5-dimethyladamantane) has been used clinically for the treatment of Parkinson's disease. It has been reported recently that memantine have antagonistic effect at the NMDA type of glutamate receptor. Although numerous studies have reported the NMDA antagonistic properties of memantine, there are no data on neurotoxic effects of memantine in vivo. To investigate the effect of memantine in the CNS, the experiments were designed to examine the neurotoxic potential of memantine using the induction of HSP-70 as a marker of neuronal damage. Memantine (10-75 mg/kg. i.p.) was injected into male SD rats. The immunochemistry of HSP-70 was studied 24 hr after administration of memantine. The administration of memantine produced the HSP-70 in the posterior cingulate and retrosplenial cortex of rat brain, similar with other NMDA antagonists. This result suggest that memantine have antagonistic effect at NMDA receptor in vivo and raise the possibility that memantine may cause neuronal injury.

722.10

CONFOCAL IMAGING OF A GLUTAMATE RECEPTOR SUBUNIT ON LIVING HIPPOCAMPAL NEURONS S.A. Richmond[1], A.J.Irving[2], F. Michaelangelli[3], E. Molnar[4], J. McIlhinney[4], W.W. Anderson[1]*, J.M. Henley[1] and G.L. Collingridge[1] [1]Dept. of Anatomy, Medical School, University of Bristol, Bristol BS8 1TD, U.K. Depts of Pharmacology [2] and Biochemistry [3], University of Birmingham, Birmingham B15 2TT, U.K. [4] MRC Anatomical Neuropharmacology Unit, University of Oxford, Oxford 0X1 3TH, U.K.

In order to observe glutamate receptor subunits in a living system, well-characterised polyclonal antibodies directed against the extracellular N-terminus of the AMPA receptor subunit GluR1 were applied to hippocampal neurons in culture. GluR1 immunoreactivity was visualised by the addition of fluorescent secondary antibody and by confocal laser-scanning microscopy (BioRad MRC 600 U/V). On the soma and dendrites of mature neurons puncta of immunoflourescence were observed. In contrast glial cells were immunonegative. In double-labelling experiments the majority of GluR1 immunoreactivity was associated with that of synaptophysin, although some extrasynaptic labelling was also apparent. Using a technique that enables discrimination between membrane-bound and intracellular immunoreactivity, cytosolic GluR1 immunofluorescence was present predominantly in the soma of hippocampal neurons, although some intracellular immunoreactivity was also present in primary dendritic regions. In addition, a few neurons that did not express GluR1 on the plasma-membrane displayed considerable cytosolic GluR1 immunoreactivity. These results demonstrate the viability of differentiating between populations of surface and intracellular receptor subunits.

Supported by the MRC and Wellcome Trust.

722.11

SYNAPTIC AND SUBSYNAPTIC DISTRIBUTION OF GLUTAMATE RECEPTOR SUBUNITS IN CEREBRAL CORTEX. Weinberg R.J.,* Popratiloff A., Wenthold R.J.† and Kharazia V.N. Dept. Cell Biology, UNC, Chapel Hill, NC 27599; †Lab of Otolaryngology, NINDS 20892.

LM immunocytochemistry suggests that glutamate receptors concentrate at synapses. This conclusion is generally supported also by EM studies, though non-synaptic staining has also been reported. To determine more accurately the location of these receptors, a new postembedding gold technique was used to localize glutamate receptor subunits in rat S-1. Analysis of gold particles coding for AMPA receptor subunits GluR1, 2, 3 and NMDAR1 revealed that labeling concentrated over active zones, with maximal density over the synaptic cleft and postsynaptic membrane. Significant labeling was also found in presynaptic terminals, especially for NMDA receptors. Subunits were also found within dendritic cytoplasm, concentrating ~20 nm inside the postsynaptic membrane. Distribution of all four receptor subunits across the synaptic cleft was similar; however, subunits differed in their tangential distribution along the active zone: GluR1 and NMDAR1 concentrated in central parts of the active zone, whereas GluR2 was denser at its periphery.

These results demonstrate that Glu receptor subunits are mainly at synapses. Subsynaptic labeling may represent a reserve pool of receptor proteins. We hypothesize that rapid translocation of receptor from this pool into the synaptic membrane may be involved in certain types of synaptic plasticity. Supported by NIH # NS-29879 (to RJW)

722.12

SYNAPTIC LOCALIZATION OF AMPA AND NMDAR1 RECEPTOR SUBUNITS IN RAT CEREBRAL CORTEX. Kharazia V.N.*, Phend K., Wenthold R.J† and Weinberg R.J. Dept. of Cell Biology & Anatomy, UNC, Chapel Hill, NC 27599; †Laboratory of Otolaryngology, NINDS, NIH, Bethesda, MD 20892.

We used postembedding immunogold labeling to study synaptic localization of glutamate receptor subunits on dendrites of cortical neurons. In neuropil of layers II-III of S-1, AMPA-positive axospinous synapses were ten times more frequently encountered than were AMPA-positive axodendritic synapses, whereas NMDA-positive synapses were only 4 times more likely to be axospinous than axodendritic. At least 90% of synapses double-labeled for GluR1 and GluR2 were axospinous, whereas only 75% of synapses double-labeled for GluR2/3 and NMDAR1 were axospinous. Since the large majority of cortical synapses are axospinous, these data suggest that NMDA receptors are concentrated on dendritic shafts, whereas AMPA subunits are more uniformly distributed. Supporting a differential distribution of AMPA and NMDA subunits, NMDAR1 staining in layers I-IV was over smaller active zones than GluR1 or GluR2/3 staining ($p < 0.001$). Since most synapses onto GABAergic neurons are axo-dendritic, we examined material double-labeled for receptors and GABA. AMPA staining over axodendritic synapses onto GABA-positive profiles was common, but NMDA staining was rare. Thus, NMDA appears to concentrate at synapses onto dendritic shafts of pyramidal neurons. Supported by NIH # NS-29879 (to RJW)

722.13

AMPA AND NMDA RECEPTOR SUBUNITS UNDERLYING FINE CALIBER PRIMARY AFFERENTS. Popratiloff A*, Wenthold R.J.†, Weinberg R.J. and Rustioni A., Dept of Cell Biology & Anatomy, UNC, Chapel Hill, NC 27599; †Lab. of Otolaryngology, NIH, Bethesda, MD 20892.

Glutamate is the major neurotransmitter in primary afferents terminating in the dorsal horn of the spinal cord. Different classes of primary afferents may be presynaptic to different glutamate receptors. We report initial results of an EM study of the distribution of these receptors, using a new postembedding immunogold technique. Rats were perfused with mixed aldehydes; sections, postfixed in tannic acid (rather than osmic acid) and treated with iridium tetrabromide and phenylenediamine, were processed as described previously (Phend et al., 1995) using antibodies for the AMPA subunits GluR1, GluR2/3 and NMDAR1. The distribution of gold particles coding for these antigens over different types of terminals was studied. Counts from all terminals in lamina II identifiable on morphological grounds as primary afferent terminals showed that the large majority of particles were in a region between 30 nm outside, and 40 nm inside the postsynaptic membrane. Particles coding for GluR1 and GluR2/3 were almost exclusively associated with the postsynaptic membrane; particles for NMDAR1 were also present more than 30 nm presynaptic to the active zone. Preliminary results indicate that primary afferent terminals of different morphologies, likely to exhibit differing functional properties, contact the GluR1, GluR2/3, and NMDAR1 subunits in differing proportions. Terminals likely to release both glutamate and neuropeptides were more likely than others to be presynaptic to NMDA receptors.

722.14

EXPRESSION OF THE NMDAR1 GLUTAMATE RECEPTOR SUBUNIT IN COS-7 CELLS. J.E. Roth*, P.H. Franklin, T.F. Murray and M. Leid. College of Pharmacy, Oregon State University, Corvallis, OR 97331.

COS-7 cells were transiently transfected with the rat NMDAR1 (NR1) subunit and the pharmacologic properties of homomeric NR1 recombinant receptors characterized using radioligand binding techniques. In addition, transient transfection of NR1 using electroporation and calcium phophate precipitation methods were compared. A 3.5 kb HindIII - StuI fragment containing the NR1-1a coding and flanking sequences was excised from pN60 (a gift from S.Nakanishi) and inserted into the HindIII - SmaI digested pTL1 (Leid et al., Cell. 68:377, 1992) to yield the eukaryotic expression vector NR1. COS cells were cultured in Dulbecco's modified Eagle's medium and plated to a density of 5×10^5 cells/10cm plate one day prior to transfection. Cells were transiently transfected with 10 µg NR1 using a BTX ECM600 electroporation system or by calcium phosphate precipitation. Inter-plate transfection efficiency was normalized by co-transfection with pSG5-LacZ (a gift from Ph. Kastner), a high copy number β-galactosidase expression vector. Membrane homogenates derived from transiently transfected COS cells showed specific binding of the glycine site antagonist [^{3}H]5,7-dichlorokynurenic acid (20 nM) 24 hours post-transfection. Expression of NR1, as demonstrated by specific binding of [^{3}H]5,7-dichlorokynurenic acid, was enhanced ten-fold (1.8 pmol/mg protein) in COS cells transiently transfected by electroporation relative to those transfected by calcium phosphate.

This work was supported by an NIH grant to TFM (DA 07218).

722.15

GLUTAMATE RECEPTOR SUBTYPES ON DISSOCIATED MAGNOCELLULAR BASAL FOREBRAIN NEURONS IN CULTURE D.J. Waters, T.G.J. Allen, J.A. Sim & D.A. Brown* Department of Pharmacology, University College London, Gower Street, London WC1E 6BT, UK.

Glutamate is the principal fast excitatory neurotransmitter in the mammalian CNS. We have attempted to identify subtypes of glutamate receptor present on magnocellular cholinergic cells from rat basal forebrain nuclei (BFN). BFN neurons were cultured from 12-14 day-old Sprague-Dawley rats (Allen, Sim & Brown (1993) J. Physiol. 460 91-116.) After 5 days to 3 weeks in culture, magnocellular cells were voltage-clamped using the whole-cell variant of the patch-clamp technique. Responses to glutamate receptor agonists were studied by pressure-ejection from a glass micropipette or by bath-application.

Pressure-ejection of N-methyl-D-aspartate (NMDA) elicited an inward current with two components; one rapidly desensitizing and one sustained. Both components were sensitive to 1mM magnesium and to 2-amino-5-phosphonopentanoate (AP5). Pressure-ejection of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) elicited an inward current with rapidly desensitizing and sustained components. Both components were antagonised by kynurenic acid and by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Bath-application of AMPA produced a large steady-state current with an EC_{50} of approximately 3μM. Bath-application of the benzothiodiazide cyclothiazide greatly potentiated AMPA-induced current whereas the lectin concanavalin A had little effect. Pressure-ejection of the non-NMDA agonists kainate and domoate elicited a sustained inward current, which was sensitive to both kynurenic acid and CNQX. Responses to pressure-ejection of domoate were reduced, but not eliminated, by bath-application of AMPA. These data indicate the presence of an NMDA-type, an AMPA-type and possibly a kainate-type receptor on these cells.

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722.16

ONTOGENESIS OF STRIATAL AMPA RECEPTORS IN THE RAT. M.W. Jakowec*, V. Jackson-Lewis and S. Przedborski. Dept. Neurol., Columbia University, New York, NY, 10032.

Glutamate is a major component of the chemical neuroanatomy of the basal ganglia. Its neurotransmitter/neuromodulator and neurotoxic actions are mediated by a variety of receptors. Thus far, little is known about the ontogeny of these receptors in the striatum. Therefore, we have examined the distribution of the transcripts GluR1 and GluR2 which encode subunits of the non-NMDA α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor within the striatum of the developing rat brain using *in situ* hybridization histochemistry. We found that transcripts encoding the subunit GluR1 are expressed at high levels in the striatum at postnatal days 2 and 7. In contrast, they are expressed at low levels in the adult striatum. Transcripts encoding the AMPA subunit GluR2 seem to follow the same pattern in that they are expressed at a higher level in neonates than in adults. Nevertheless, the difference in the level of expression between the neonate and the adult striatum is less dramatic for GluR2 than for GluR1. These results indicate that there are fundamental phenotypic differences in the subunit composition of AMPA receptors of neonates and adults. This phenomena may play a significant role in the establishment of proper synaptic circuitry within the developing striatum as well as contribute to differences in susceptibility to injury and disease in the aging brain.

722.17

DEVELOPMENTAL STUDIES OF NMDA RECEPTOR SUBUNITS IN RAT BRAIN. **J.-H. Luo, Y.-H. Wang, T.Z. Bosy, R.P. Yasuda, B.B. Wolfe*. Dept Pharmacology Georgetown Univ. Sch. of Med., Washington,D.C. 20007**

Developmental profiles of NMDA R1, R2A, and R2B subunits were determined in rat CNS. Quantitative immunoblots were used to determine protein levels. In all brain areas measured, levels of NR1 subunit increased 2.5-4.5 fold during the first three weeks after birth. There were large differences in the amounts (pmol/mg) of NR1 subunit expressed (2.5, HP; 1.3, CX; 1.0, OB; 0.85, MB; 0.41, CB). For NR2A, low levels were expressed in newborns followed by a progressive increase of this subunit from P7 to P22 at which time adult levels were reached. The ontogenic profiles of NR1 and NR2A were similar to each other and correspond with mRNA expression studies. Furthermore, the absolute numbers obtained were close to those reported for [3H]MK-801 binding site densities. In contrast, ontogenic profiles of NR2B subunit were distinct in all brain regions. Typically, NR2B was expressed at relative high levels at early ages (P2~P4) which sometimes increased modestly over the next several days and then decreased. Peak densities of NR2B subunit in various brain regions were attained at different times (P4, CB; P7, CX; P11, MB; P16, HP). In OB, the levels of NR2B subunit were almost constant over the entire period tested. In CB, NR2B declined after P7 to undetectable levels at P22. These data provide a rationale to explain functional changes in NMDA receptor properties in different brain regions during development. (Supported by NS28130, AG09973, AG09884)

GABA RECEPTORS V

723.1

REGULATION OF $GABA_A$ α1 SUBUNITS, NEURONAL GROWTH AND SURVIVAL BY $GABA_A$ RECEPTOR LIGANDS IN EMBRYONIC RAT BRAINSTEM CULTURES. J. Liu*[1], A.L.Morrow[2] and J.M.Lauder[1]. [1]Dept. of Cell Biology and Anat., [2]Dept. of Psychiatry, Univ. of N. Carolina, Chapel Hill, NC 27599.

Previous studies have indicated the presence of functional α, β, γ and δ subunits of $GABA_A$ receptors in embryonic rat brainstem cultures. GABA or $GABA_A$ antagonist bicuculline differentially regulate the survival of brainstem monoamine and GABA neurons (Liu et al., 1994). In the current study, dissociated cultures were prepared from embryonic day 14 (E14) brainstem and cultured in BME + 10% NuSerum for 1 day *in vitro* (DIV) , then treated for 48 hrs with 10 μM GABA and /or 10 μM bicuculline or the organochlorine pesticide dieldrin in serum-free medium. Effects of GABAergic agents on relative levels of α1 protein were determined by quantitative immunobinding assays and absolute amount of α1 mRNA transcripts were quantified by competitive PCR. Results indicated that expression of α1 protein and mRNA were up-regulated by both bicuculline and dieldrin. Effects on survival of neurons were quantified by counts of immunoreactive cells. GABA promoted survival of 5-HT and TH neurons. Dieldrin blocked this effect, and by itself had inhibitory effects on survival of these cells and stimulatory effects on survival of GABA neurons. Cell size, shape and complexity of neurite outgrowth by 5-HT, TH and GABA neurons were analyzed by computer-assisted morphometry. GABA, bicuculline and dieldrin also had differential effects on neurite outgrowth by these neurons depending on their neurotransmitter phenotype. These results support the hypothesis that GABA is an important trophic signal for developing monoamine neurons and may regulate expression of its own receptors during brain development. $GABA_A$ antagonists, such as organochlorine pesticides may interfere with this regulation. Supported by NIEHS grant to JML. The authors thank Dennis Grayson for providing α1 internal standards for competitive PCR.

723.2

$GABA_A$ RECEPTOR SUBUNIT mRNA PROFILES IN SINGLE DEVELOPING CEREBELLAR GRANULE CELLS. E. V. Grigorenko*, T. Y. Rikhter and H. H. Yeh. Dept. Physiology & Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157

Although $GABA_A$ receptor ($GABA_AR$) subunits are differentially expressed during brain development, the changes in subunit profiles remain to be analyzed for individual neurons. We are examining the molecular profiles of $GABA_AR$ subunits in the postnatal cerebellum and in single cerebellar granular cells developing *in situ* and in culture.

In whole cerebellar tissue, prominent expression of the α1, β2 and γ2S subunits were detected by birth and remained relatively constant throughout the first two postnatal weeks. Other subunits tended to increase either progressively (γ2L) or abruptly (e.g. α6 and δ) around the time of formation of the granule cell layer. Granule cells derived from postnatal day 8 cerebellum were also maintained in long term cultures. Single cells maintained for 4, 9 and 13 days in culture (DIC) were harvested and profiled for representative messages normally expressed in mature granule cells, i.e., α1, α6, β2, β3, γ2S and δ. To date, the α1 and β2 messages have been detected by 4 DIC, and α6 by 9 DIC. The abrupt increase in the expression of the δ subunit message was observed around 7 DIC.

Our data indicate that $GABA_AR$ subunit genes are differentially and developmentally regulated. The demonstration at the single cell level carries the analysis to identified individual developing neurons, providing prerequisite information for a systematic developmental and functional analysis of $GABA_AR$ expression in the cerebellum.

723.3

GABA$_A$ CURRENTS IN DEVELOPING MEDIAL SEPTUM/DIAGONAL BAND (MS/DB) NEURONS. S.-H. Hsiao[1], J.R. West[2] & G.D. Frye[1]*. Dept. of Med. Pharmacol. & Toxicol.[1], Dept. of Med. Anat.[2], Texas A&M Univ. Col. of Med., College Station, TX 77843-1114

The GABA$_A$ receptor is a ligand-gated Cl^- channel which plays a prominent inhibitory role in the adult central nervous system. During postnatal development, the expression of GABA$_A$ receptor subunits undergoes major changes (Laurie et al., J. Neurosci. 12:4151, 1992) and receptor function may shift from mediating excitation to inhibition (Gaiarsa et al., J. Neurobiol. 26:339, 1995). The present study examined changes in GABA$_A$ currents in acutely dissociated neurons from MS/DB of postnatal male and female Sprague Dawley rats. Two age groups of rats were examined: pups (pn4-10) and juveniles (pn11-16). GABA (0.3-300 μM) response curves were concentration- and development-dependent. The maximum GABA response of the juvenile group (338.7±66.4 pA/pF, n=12) was significantly larger than that of pups (176.8±25.8 pA/pF, n=11; p>0.05). The Ka values changed as well (7.66±1.50 μM juveniles vs 3.64±0.71 μM pups; p<0.05). The Hill coefficient (1.71±0.11 vs 1.86±0.16; p>0.05) and capacitance (16.12±1.47 vs 12.95±1.26; p>0.05) did not change for juveniles vs pups, respectively. These data suggest that there are significant changes in GABA$_A$ receptor function in MS/DB neurons during early development in the rat. Supported in part by AA06322 (GDF).

723.4

DEVELOPMENTAL INDUCTION OF GABA$_A$ RECEPTOR α1-SUBUNIT POLYPEPTIDES IN CHICK EMBRYO CORTEX AND DERIVED NEURONS IN CULTURE. J.D. Miranda* and E.M. Barnes, Jr. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

In order to produce high-titer antibodies which are capable of recognizing native GABA$_A$ receptor subunits, we have produced fusion proteins containing selective receptor sequences from the intracellular loop region. The chick GABA$_A$ receptor α1(331-381) subunit sequence was expressed as a fusion protein containing a hexahistidyl leader peptide, purified by Ni^{2+}-affinity chromatography, and used for rabbit immunizations. The resulting RP4 antiserum showed a high titer for immunoprecipitation of receptor ligand binding to cortical extracts from chick embryos 16 days in ovo. Representing the threshold for antibody saturation, 1 μl of crude antiserum was able to immunopreciptate 61% of ^{3}H-flunitrazepam binding. The antiserum is also selective because no cross reaction was detected on slot blots of β2S(317-428) or β4S(316-437) fusion proteins. Western blots utilizing a 1:1000 dilution of the RP4 immune serum showed a reaction with a single 50-51 kDa polypeptide from chick embryo cortex. This protein was not detected in heart, liver or skeletal muscle. Similar analysis on immunoblots revealed an increase in the expression of the GABA$_A$ receptor α1-polypeptide in chick cortical neurons from day 2 to day 6 in culture and in chick cerebral cortex from embryonic to adult stages. In both cases this pattern of developmental expression was similar to that found previously for induction of the major ligand binding sites of GABA$_A$ receptors.

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723.5

A RHO-LIKE GABA RECEPTOR SUBUNIT IS TRANSIENTLY EXPRESSED IN NEONATAL RAT BRAIN. M.Mladinic,E.Cherubini*.Biophys.Lab.,Int.Sch. Adv. Studies (SISSA), 34013Trieste, Italy.

A novel bicuculline and baclofen insensitive chloride mediated response has been described in neonatal hippocampus (Strata&Cherubini, J.Physiol.480,493-503,1994).This response clearly resembles the one present in the retina and supposed to be mediated by GABA$_C$ receptors. It is conceivable that this new receptor channel is an heterooligomeric protein assembled from the rho1 subunit.To see whether a rho-like GABA receptor subunit is present in the rat brain, *in situ* hybridization on brain sections from neonatal and adult rats was performed. Rho1 and rho2 GABA receptor subunit specific antisense oligonucleotide probes as well as another probe that revealed both rho1 and rho2 subunits (rho1+rho2) have been used. The rho1 probe was homologous to the 1325-1372 nucleotide sequence of the rho1 gene (Cutting et al., Proc.Natl.Acad.Sci.USA, 88,2675,1991) and the rho2 probe to the 1249-1296 nucleotide sequence of the rho2 gene (Cutting et al.,Genomics,12,804,1992). The rho1+rho2 probe was homologous to the nucleotide sequence of the rho1 gene (1130-1174) and nucleotide sequence of the rho2 gene (1042-1086). As a positive control a beta3 subunit specific antisense probe was used. Negative control were sense oligonucleotide probes. The same probes were utilized to hybridize sections of the rat retina. The autoradiographic signals obtained with rho1 and rho2 probes were low in the neonatal as well as in the adult brain. However signal obtained with rho1+rho2 probe was suprisingly strong in the neonatal brain sections, expecially in the forebrain or in the hippocampus. In the retina high signals with all three rho probes were obtained.In the adult brain the signal obtained with rho1+rho2 probe was present only in the cerebellum but comparing to neonatal brain was lower.These results strongly suggest that a novel rho-like subunit is expressed in the neonatal brain during a critical period of postnatal development.

723.6

DIFFERENTIAL INTERACTION BETWEEN NEUROACTIVE STEROIDS AND DIAZEPAM AT THE GABA$_A$ RECEPTOR IN RAT FETAL VS ADULT BRAIN. C.K. Kellogg* and G.L. Pleger. Dept. of Psychology, Univ. of Rochester, Rochester, New York, 14627.

Specific neuroactive steroids elicit positive modulatory effects on the GABA$_A$ receptor. This observation, coupled with our observations that prenatal exposure to diazepam (DZ) alters neural and behavioral stress responses that emerge over adolescent development under the influence of gonadal steroids, led us to hypothesize that neuroactive steroids may exert organizational influences during development via the GABA$_A$ receptor. Early exposure to DZ could alter this influence. Measuring GABA-stimulated ^{36}chloride influx in synaptoneurosomes prepared from the cerebral cortex of adult males and fetal forebrain at 20 days gestation. we evaluated the interaction between DZ and neuroactive steroids that affect the GABA$_A$ receptor and that could be present in fetal tissue during gestation. The 3α,5β progesterone metabolite, pregnanolone (P, 500 nM), increased the potency (decreased the EC$_{50}$) of GABA in both adult male and mixed-sex fetal tissue by 38-48%. DZ also increased the potency a comparable amount in adults, but the effect was much more pronounced in fetal tissue (a 77% decrease). The in vitro addition of both DZ and P had an additive effect in the adult but in fetal tissue, the potent effect of DZ masked an effect of P. The testosterone metabolite, androsterone (2.5 μM), also decreased the EC$_{50}$ in adult and fetal tissue (separated by sex), but the effect was more pronounced in fetal tissue, particularly in female forebrain. Supported by Grant No. DA 07080.

723.7

PRENATAL DIAZEPAM EXPOSURE: INFLUENCE ON BASAL AND STRESSOR-INDUCED mRNA LEVELS FOR GABA$_A$ RECEPTOR SUBUNITS. A.A. Roberts*, M.H. McCollum, G.L. Pleger, and C.K. Kellogg Dept. of Psychology, Univ. of Rochester, Rochester, New York, 14627.

Developmental modulation of GABA$_A$ receptor function, via administration of diazepam (DZ) to the pregnant dam, alters behavioral and GABA$_A$ receptor responsiveness to stressors in the exposed adult rat. Recent studies have also shown that the molecular composition of the GABA$_A$ receptor is linked to pharmacologic responsiveness of the receptor. Furthermore, exposure to acute environmental challenges changes mRNA levels of specific receptor subunits. In this study, we evaluate the effect of exposure to DZ (2.5 mg/kg) over gestational days 14-20 on basal mRNA levels for α_1 and α_2 GABA$_A$ receptor subunits in the cerebral cortex, hippocampus, and hypothalamus of adult (60-70 days) male rats. The results are compared to those from animals exposed over the same period to vehicle (40% propylene glycol, 10% ethanol) or co-exposed to DZ and the antagonist flumazenil (10 mg/kg). Early exposure led to a significant 15% increase in the basal level of mRNA for α_1 in the cerebral cortex. This effect of DZ was prevented by co-exposure to flumazenil. There was no change in α_1 mRNA in any other region, and α_2 mRNA was unaffected in all regions examined. Because early DZ exposure interferes with environment-specific social behavior, we have examined the effect of this challenge on mRNA for the two subunits. In naive adult male rats, cortical mRNA for α1 is significantly increased by 10 min of testing in either a familiar or novel environment. The impact of early DZ exposure on this response is being evaluated. Supported by Grant No. DA 07080.

723.8

GABA$_A$-Cl RECEPTOR COMPLEX IS INVOLVED IN SEXUAL DIFFERENTIATION OF PARENTAL BEHAVIOR.

Del Cerro, M.C.R. (3); Pérez-Laso, C.; Rodríguez-Zafra, M.; Ortega, E. (2); Claro, F.; Ambrosio, E.; Izquierdo, M.A.P.; Guillamón, A. and Segovia, S. (1) . Departamento de Psicobiología, Ciudad Universitaria s/n, U.N.E.D. 28040 MADRID. SPAIN. (2) Departamento de Bioquímica y Biología Molecular, Facultad de Medicina. Universidad de Granada. GRANADA. SPAIN.*

In this work we study the effects of experimentally induced changes in the opening frequency of Cl^- channel of the GABA$_A$/BDZ receptor complex on the sexual differentiation of maternal behavior (MB) and its correlation with morphological and/or endocrine alterations. Our rationale is as follows: 1) MB is a sexually dimorphic behavior; 2) MB is under hormonal control and tonic vomeronasal inhibition; 3) The vomeronasal system (VNS) is a sexually dimorphic neural system; 4) GABA is present in about 90% of the accessory olfactory bulb (AOB) granular populations; 5) Diazepam (DZ) postnatal injections during neural sexual differentiation period induce a feminine morphological pattern of the AOB (volume and neuron numbers)in the male rat and facilitates the induction of MB in virgin females when adult; 6) DZ increases the the opening frequency of the Cl^- channel of the GABA$_A$/BDZ receptor, while Picrotoxin (PIC) reduces it.

We hypothesize that treatment during developmental periods with drugs acting in an opossite way on the GABA$_A$/BDZ Cl^- channel (DZ/PIC), would cause opossite behavioral, morphological and/or endocrine effects.

Fifty rat pups of the Wistar strain were randomly divided in the day of birth into four groups: a) untreated males (UT males N=15); b) 2.5 mg/Kg of DZ s.c. injected males (DZmales N=10); c) untreated females (UT females N=15) and d) .5 mg/Kg PIC s.c. injected females (PIC females N=10). MB test was initiated at 90 days of age, during 12 consecutive days. MBR Software was used to register MB. Blood samples were collected before transcardiac perfusion, AOB removed and histologically processed.

DZ treatment significantly increased the following MB patterns in relation to UT males and PIC females: Nest score, grooming, licking and physical contact to pups and percentage of animals reaching sensitization criteria (2 consec. days of complete retrieval). DZ males did not differ from UT females in any behavioral pattern, except in licking.

Our results indicate that Cl^- channel is involved in the sexual differentiation of the VNS.

This work hasbeen supported by DGICYT PM91-027 (2) and PB93-0291-C03-=3 (3).

723.9

LOSS OF Na,K-ATPase α3-ISOFORM mRNA EXPRESSION IN THE INTERNEURONS OF AGING RAT HIPPOCAMPUS. N. B. Chauhan* and G. J. Siegel. Cellular and Molecular Neuroscience, Department of Neurology, Hines VA/Loyola University Chicago, , Hines, IL 60141.

Age-related changes in the expression of Na,K-ATPase α1- and α-3 isoform mRNA in young (3 month, N=6) and old (24 month, N=6) Fischer-344 male rat brains were analyzed. *In situ* hybridization was performed on 5 µm-thick paraffin sections of paraformaldehyde-perfused brains with [^{35}S] labeled riboprobes. Sections were hybridized for 21 h at 42°C, treated with RNase A, washed and exposed to NTB-2 Kodak emulsion for 4 weeks at 4°C. After development and counterstaining with cresyl violet, grains were counted under dark-field illumination with the use of the BioQuant Image Analyzer. Sense controls showed no significant labeling. Total grains were counted in 10 squares of 250^2 µm^2 in each of stratum moleculare (SM), stratum radiatum (SR) and stratum oriens (SO), and over the single interneurons within each square. The results show: (1) Specific labeling of α3-mRNA in the young rat over medium- and large-sized interneurons within SM, SR and SO in the form of grain clusters. (2) In addition, fine grains related to α1- and α3-mRNA are distributed diffusely throughout SM, SR and SO. (3) Aging resulted in more than 95% loss of α3-mRNA clusters over the interneurons within SM, SR and SO, with no significant change in the cell density. (4) The diffuse fine grain density for α3-mRNA, excluding interneurons, in the old rat did not show any significant change relative to the young ($P>0.05$) (ANOVA, unpaired, two-tailed Student-*t* test). (5) The diffuse density of α1-mRNA grains in SM, SR and SO increased by 8 to 10 times in the old rats ($P<0.0001$). The increased α1-mRNA may be due to age-related glial hyperactivity. The loss of neuron specific α3-mRNA in the GABAergic interneurons may reflect an early change in GABA-mediated inhibition underlying age-related memory and cognitive deficits. (Supported by Rush Alzheimer's Disease Center and Pott's Foundation, Loyola University)

723.10

Multiple Kinases differentially modulate GABAergic conductances in cultured retinal neurons E.Wexler*, P.K.Stanton, S.Nawy; Depts. Neuroscience, Neurology and Ophthalmology and Visual Sciences,

GABA gated chloride channels have been found to be present in all types of retinal neurons. The most thoroughly studied are those belonging to the classically defined "GABAa" subtype. The responses mediated by these receptors are characterized by a rapid, non-voltage dependent desensitization and competitive antagonism by bicuculline. Studies by other investigators have demonstrated the presence of multiple consensus sites for phosphorylation by a variety of protein kinases. The effects of these kinases upon GABAergic responses were investigated in cultures of retinal ganglion cells using fast flow perfusion and conventional whole-cell recording techniques. In agreement with (Veruki and Yeh 1994), we find that the application of Vasoactive Intestinal Peptide (1µM), which is to known activate adenylate cyclase, transiently potentiates GABA evoked responses in ganglion and bipolar cells. Either Phorbol-Diacetate (10µM) or Phorbol-DiButyrate (1µM), in the intracelluar pipette solution did not alter the rate at which GABA responses rundown. In order to examine the effect of PKG mediated phosphorylation we perfused extracellularly with a cell permeant cGMP analogue known to activate PKG. One minute application of either 8Br-cGMP 1 mM resulted in a 15% decline in the GABA evoked response as compared to 2.6% for controls. In sum, ganglion cells are able to fine tune their response to GABA by selective activation of different protein kinases. Supported by NIH grant EY-10254, and ALCON Laboratories.

723.11

POSSIBLE ALTERATIONS IN [^{35}S]t-BUTYLBICYCLOPHOSPHOROTHIONATE (TBPS) BINDING AFTER *IN VITRO* PHOSPHORYLATION OF $GABA_A$ RECEPTOR: AN AUTORADIOGRAPHIC STUDY T. Ito*, T. Suzuki, S. E. Wellman and I.K. Ho. Dept. of Pharmacol. & Toxicol., Univ.of Mississippi Med. Ctr., Jackson, MS 39216

Increasing reports on intracellular regulation of the $GABA_A$ receptor show possible involvement of phosphorylation/dephosphorylation of the receptor. Consensus sequences for protein kinase(s) are inferred from some subunit DNA sequences. Electrophysiological studies reveal that desensitization and/or "run-down" of $GABA_A$ receptors may be related to their state of phosphorylation. In this study, alterations in $GABA_A$ receptors caused by phosphorylation were visualized using receptor autoradiography. Frozen rat brain slices were treated with 50 µM Mg-ATP at physiologically relevant conditions after preincubation in 50 mM phosphate buffer with 200 mM NaCl and 1 mM EDTA. Treated slices were then incubated in the phosphate buffer with 3 nM [^{35}S]TBPS. Major alterations were observed in the cerebellum. Binding was decreased in the granule cell layer (-39% of control), while that in the molecular layer showed an increase(+26% of control). Because the α_6 subunit is localized to the granule cell layer, the observed increase maybe related to this subunit. Moreover, since the mean changes in binding were in either direction, the effect of phosphorylation might be region-specific. Although this is an indirect assay for receptor phosphorylation, our method detected region-specific alterations that biochemical studies can not detect. It may be a powerful and versatile tool for studies of receptor phosphorylation for other receptors as well as for $GABA_A$ receptors. (supported by NIDA-04480)

723.12

PHOSPHATASE INHIBITORS SHORTEN IPSC DECAY BY ALTERING $GABA_A$ CHANNEL KINETICS.

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Phosphorylation and dephosphorylation are potentially important mechanisms for regulating the shape of inhibitory postsynaptic currents (IPSCs), but have usually been studied with long applications of low GABA concentrations. The synaptic GABA transient, however, is thought to be quite high and brief. Therefore, we studied the effects of phosphatase inhibitors on the decay of IPSCs, and on chloride currents activated by brief (2-10 ms) pulses of saturating (1-10 mM) GABA to outside-out patches.

Cultured rat hippocampal neurons and patches were voltage clamped (-60 mV, 25°C) using Cs or KCl-based pipette solutions (5 mM ATP, ~50 nM free Ca^{2+} with BAPTA). We evoked autaptic IPSCs with 5 ms steps to +60 mV. A piezoelectric device was used to exchange control and GABA-containing solutions in patch experiments. Whole-cell recordings were held for >200 seconds before pulling patches when phosphatase inhibitors or ATPγS were present in the patch pipette. All current decays were best fit with two exponentials, but for simplicity are expressed as an average time constant: $\bar{\tau} = (Amp_1 \times \tau_1) + (Amp_2 \times \tau_2)$.

ATPγS (5 mM) significantly ($p < 0.05$) shortened *IPSC* decay from 89 ± 32 ms (n = 4, mean ± SD) to 39 ± 9 (n = 3), as did the serine/threonine phosphatase inhibitors calcineurin inhibitory peptide (CIP, 300 µM, 32 ± 8, n = 5) or okadaic acid (OA, 5 µM, 45 ± 12, n = 6). ATPγS, CIP and OA also significantly shortened the patch current decay from 122 ± 40 ms (n = 13) to 45 ± 11 (ATPγS, n = 9), 64 ± 20 (CIP, n = 4) and 52 ± 26 (OA, n = 4). These results suggest that under synaptically relevant stimulation, prolonged $GABA_A$ channel gating requires serine/threonine phosphatase activity. Dynamic regulation of endogenous phosphatase activity may thus produce >2-fold changes in the duration of IPSCs.

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723.13

NOREPINPHRINE AND cAMP ENHANCE DENTATE GRANULE CELL $GABA_A$ RECEPTOR CURRENTS Jaideep Kapur* and Robert L Macdonald Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104.

There is an association of noradrenergic and GABAergic terminals on the perikarya and dendrites of dentate granule cells. The effect of noradrenergic stimulation on $GABA_A$ receptor (GABAR) currents in dentate granule cells is not known.

Dentate granule cells were acutely isolated and GABAR currents were recorded using conventional whole cell-patch clamp methods. Drugs were applied by rapid application system.

500 µM or 1 mM norepinephrine and the selective β-adrenergic agonist, isoproterenol enhanced GABAR currents elicited by 30 µM or 1 mM GABA in 12/19 granule cells. 1 mM 8-Bromo - cAMP also consistently enhanced dentate granule cell GABAR currents elicited by 30 µM or 1mM GABA. The effect of cAMP on GABA concentration response relationship was studied and the data were fitted to a sigmoid dose response curve. Maximal current was enhanced in all cells (42% +/-12% N = 4, p = 0.04) . The EC_{50} shifted to the left in 2 of 4 cells, from 99 µM to 20 µM (p = 0.28). We wished to test if norepinephrine and cAMP were acting by activating cAMP-dependent kinase (PKA). GABA concentration response curves were obtained in dentate granule cell populations with intracellular recording solution containing no ATP (Maximal I_{GABA} = 174 pA, EC_{50} = 84.2 µM, n = 7), ATP regeneration system (Maximal I_{GABA} = 531 pA, EC_{50} = 41.3 µM, n = 7) or catalytic subunit of PKA and ATP regeneration system (Maximal I_{GABA}= 712 pA, EC_{50} = 20 µM, n = 4).

Norepinephrine, Isoproterenol, cAMP, PKA and ATP consistently enhanced maximal dentate granule cell GABAR currents, and produced a left shift of GABAR concentration response curve.

723.14

GABA RECEPTOR FUNCTION IS MAINTAINED BY ADENINE NUCLEOTIDES IN ACUTELY DISSOCIATED COCKROACH NEURONS. G.B. Watson* and V.L. Salgado. Insect Management Biochemistry, DowElanco, Indianapolis, IN 46268.

While vertebrate GABA receptors have been shown to be regulated intracellularly by phosphorylation, little is known about the intracellular regulation of insect GABA receptor function. Toward this end, small diameter (< 25µ) neurons from the sixth abdominal ganglion of the American cockroach, *Periplaneta americana*, were enzymatically isolated and responses to exogenously applied GABA were recorded using the whole-cell patch clamp technique. With a minimal intracellular medium, responses to repeated applications of GABA decreased to zero within a few minutes. The rate of rundown of GABA responses was decreased by the phosphatase inhibitors microcystin and okadaic acid, and accelerated by the inclusion of protein kinase A inhibitor (PKI) in the intracellular solution, strongly suggesting that phosphorylation is necessary for the maintenance of cockroach GABA receptor function.

When added to the intracellular medium, 5 mM ATP completely blocked GABA response rundown. ADP also slowed GABA response rundown, but responses stabilized at a level about half that seen with ATP. In the presence of PKI, ATP was only as efficacious as ADP in slowing rundown. PKI had no effect on the ability of ADP to slow rundown, suggesting that the β-phosphate of ADP is not involved in PKA-dependent phosphorylation of the GABA receptor. Guanine nucleotides had no demonstrable effect on GABA responses. These results suggest that in cockroach neurons, GABA receptor function is maintained intracellularly by adenine nucleotides, not only by phosphorylation, but also by an interaction with a nucleotide recognition site unrelated to PKA-dependent phosphorylation.

723.15

cAMP-DEPENDENT PROTEIN KINASE MODULATES THE FUNCTION OF RECOMBINANT GABA$_A$ RECEPTORS IN MOUSE L929 FIBROBLASTS.

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Many studies have demonstrated that phosphorylation modulates the function of ligand-gated ion channels; however, the effects of phosphorylation on the γ-aminobutyric acid type A receptor (GABAR) remain controversial. In the present study, we examined the effects of cAMP-dependent protein kinase (PKA) on the function of recombinant rat $\alpha_1\beta_1\gamma_{2L}$ GABARs expressed in L929 mouse fibroblast cells. GABAR currents were recorded using conventional whole-cell patch-clamp methods with symmetrical chloride concentrations at holding potentials of -75 mV. Complete GABA concentration response curves (1 μM- 300 μM) were obtained for each condition examined. To reduce the variability often encountered when comparing populations of cells, a reimpalement protocol was used in which paired whole-cell recordings were obtained successively from individual cells. When a standard intrapipette solution was used for both the initial and reimpalement cell recordings, GABAR peak currents (100-3600 pA) uniformly decreased (-29%, n=5) with minimal change in EC_{50} (9.3 μM to 7.5 μM). In contrast, PKA catalytic subunit applied intracellularly on reimpalement enhanced peak current amplitudes when compared to the initial control impalement (+9% , n=13), with no significant shift in GABA EC_{50} (14.1 μM to 15.1 μM). This enhancement was not observed if protein kinase inhibitory peptide (PKIP), a specific PKA inhibitor, was coapplied with PKA in the recording pipette on reimpalement (-9%, n=5). A similar decline in peak current amplitudes was seen on reimpalement with PKA in cells expressing recombinant receptors containing the β_1 subunit mutation of serine 409 to alanine (-12%, n=6), a consensus site for PKA phosphorylation in the putative cytoplasmic domain. These data indicate that PKA phosphorylation of the β_1 subunit at a specific serine residue modulates GABAR responses. Support by R01-NS03300 (RLM), T32NS07222 (KFH).

723.16

PROTEIN KINASE C MODULATES GLYCINE-ACTIVATED CHLORIDE CURRENTS OF HYPOTHALAMIC NEURONS.

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Glycine (Gly) is an important inhibitory transmitter in the spinal cord and other regions of the CNS. While the modulatory influence of phosphorylation by protein kinase C (PKC) on the Gly receptor has been explored for oocytes injected with either poly(A)+ mRNA isolated from nervous tissue or synthetic mRNA, little is known about the influence of PKC on the native Gly receptor protein of intact cells. Therefore, we examined the effect of phorbol dibutyrate (PDBu), an activator of PKC, on Gly-activated current, IGly, of hypothalamic neurons freshly isolated from the brains of young mice. IGly was recorded with the nystatin perforated patch technique in order to minimize perturbation of the cytoplasmic milieu that occurs with conventional whole cell recording. Bath application of 1 μM PDBu consistently depressed IGly; for 7 separate neurons, peak IGly declined to 36.6 ± 15.5 % of control. Although the time course of this effect was variable, peak suppression occurred within 2-5 min after PDBu. For some neurons, the suppression of IGly persisted while for others it recovered even in the continued presence of PDBu. These results suggest that the Gly receptor can be phosphorylated *in vivo* by PKC. Thus, neurotransmitters affecting the activity of PKC could profoundly alter the efficacy of Gly as an inhibitory neuromediator in the CNS. NIAAA AA08025 and NIH NS31040.

723.17

THE ALPHA SUBUNIT OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II ENHANCES GABA$_A$ AND GLYCINE RECEPTORS-MEDIATED RESPONSES OF SPINAL NEURONS.

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Evidence exists for the regulation of γ-aminobutyric acid type A (GABA$_A$) and glycine receptors function by protein kinase A and protein kinase C. In addition, phosphorylation of GABA$_A$ receptor by calcium/calmodulin-dependent protein kinase II (CaM-KII) has been recently demonstrated (*J. Biol. Chem.* 269:18111, 1994), suggesting a role for this kinase in modulating GABA$_A$ receptor function *in vivo*. Here we report that in acutely isolated rat spinal dorsal horn (DH) neurons, intracellularly applied the α-subunit of CaM-KII increased GABA (5-20μM)- and muscimol (2-5μM)-activated currents (GABA: to 164.2% ± 10.3 of control; n=14; muscimol: to 150.6% ± 6.3, n=4) recorded with the whole-cell patch-clamp technique. This effect was associated with reduced desensitization of the GABA response. Heat-inactivated CaM-KII (n=12) did not alter the GABA responses. In addition, GABA$_A$-mediated inhibitory postsynaptic potentials in rat hippocampal CA1 neurons were enhanced by CaM-KII (to 204.4% ± 29.8, n=6). Since GABA and glycine coexist in the DH, we examined the possibility of modulation of glycine-activated current in DH neurons by CaM-KII. The α subunit of CaM-KII increased (to 180.7% ± 22.9, n=7) glycine-induced current, whereas heat-inactivated CaM-KII did not alter (to 109.3% ± 15.8, n=8) the glycine response. These results suggest that the function of GABA$_A$ and glycine receptors is enhanced by the α subunit of CaM-KII, either by direct CaM-KII phosphorylation of the GABA$_A$ and glycine receptors, or indirectly through phosphorylation of some membrane-bound regulatory proteins. This mechanism may contribute to long-term enhancement of inhibitory synaptic transmission. (Supported by NS-26352 and IBN-9209462).

723.18

REGULATION OF γ-AMINOBUTYRIC ACID TYPE A (GABA$_A$) RECEPTORS BY PROTEIN PHOSPHORYLATION.

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We have investigated the role of a number of protein serine/threonine and tyrosine kinases in modulating the function of GABA$_A$ receptors.

All β receptor subunits are phosphorylated on a conserved serine residue by PKA, PKG, PKC and CamKII, the β1 and β 3 subunits also contain a second site that is phosphorylated by CamKII only. Both γ2 subunit types (γ2S and γ2L) are phosphorylated by PKC and CamKII,the γ2L subunit having an extra recognition site for both kinases. Receptor phosphorylation by protein serine/threonine kinases reduces the response to applied GABA, the magnitude of this effect being dependent on subunit composition.

Coexpression of protein tyrosine kinase vSRC with receptor subunits results in specific phosphorylation the β1, γ 2S and γ2L subunits. Receptor tyrosine phosphorylation by vSRC enhanced the response to applied GABA.

Our results suggest that GABA$_A$ receptors may be functionally up-regulated or down-regulated via a number of signal transduction pathways which may have profound effects on inhibitory transmission in the nervous system.

723.19

AMINO ACID RECEPTOR EXPRESSION IN DEVELOPING HYPOTHALAMUS: GABA EVOKES LARGE CURRENTS AT EARLY EMBRYONIC AGE.

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Whole cell voltage and current clamp were employed to investigate the developmental changes of GABA, glutamate, and glycine responses in cultured embryonic neurons (N=140) from rat hypothalamus. Every neuron studied from a few hours after plating at E15 to two weeks later responded to GABA (30 μM). The currents evoked by GABA increased by 5-fold within two weeks in culture. The time constants of the desensitization of GABA currents did not change during this period. In younger cultures, GABA (5 μM) induced depolarization and action potentials rather than hyperpolarization in gramicidin perforated current clamp recordings. Glutamate and glycine mediated currents developed later than GABA mediated currents. Whereas all neurons expressed GABA receptors, some developing neurons did not express glutamate receptors or glycine receptors. The percentage of cells responding to glutamate (500 μM) or glycine (500 μM) was 20% at 0 days in vitro (DIV), and increased to 100% at 6 DIV. The glutamate and glycine mediated currents increased by 20- and 50-fold within two weeks in culture. Astrocyte substrates increased the amplitude of currents evoked by and the percentage of cells responding to both glutamate and glycine, but had no effect on GABA mediated currents. The currents and conductances elicited by GABA were much greater than those generated by glutamate or glycine throughout the period examined, particularly evident in younger cells. Each of the three amino acid evoked currents increased from E15 (1 DIV) to E20 (1 DIV), indicating an intrinsic development in the expression of the amino acid receptors in vivo. The similarity of the GABA and glutamate evoked currents between E15, 10 DIV and E20, 5 DIV (both 25 days after conception) suggests parallel developmental patterns for amino acid receptor expression in vitro and in vivo. These data suggest that GABA may play a dominant role, possibly an excitatory one, in early development and that glutamate and glycine receptors may be more sensitive to regulation by the cellular environment than GABA receptors.

723.20

OPTICAL RECORDING USING A VOLTAGE-SENSITIVE DYE PROVIDES EVIDENCE FOR A NOVEL TYPE OF GABA RESPONSE IN THE EARLY EMBRYONIC CHICK BRAINSTEM.

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Evidence for a novel γ -aminobutyric acid (GABA) response was found for glutaminergic excitatory postsynaptic potentials in the early embryonic chick nucleus tractus solitarius (NTS), using a multiple-site optical recording technique employing a fast voltage-sensitive dye. In various areas of the vertebrate central nervous system, it is known that release of GABA provides inhibitory modulation of excitatory postsynaptic potentials. Classically, two classes of GABA receptors, GABA$_A$ and GABA$_B$, have been identified. The GABA$_A$ receptors are blocked by the competitive antagonist bicuculline, and the non-competitive blocker picrotoxin. The GABA$_B$ receptors are specifically blocked by 2-hydroxysaclofen. In addition, bicuculline- and 2-hysroxysaclofen-insensitive GABA$_C$ receptors have been described. The GABA response found in the present experiment was insensitive to GABA$_A$ antagonists (picrotoxin, dieldrin, bicuculline, SR95531) and GABA$_B$ antagonists (2-hydroxysaclofen, phaclofen, CGP35348), but was stimulated by either muscimol or baclofen.

723.21

THE $GABA_A$ RECEPTOR β4 SUBUNIT IS AN EMBRYONIC ISOFORM IN THE CHICK CEREBRAL CORTEX. M.H. Jalilian Tehrani*, B.J. Baumgartner, and E.M. Barnes, Jr., Dept. of Biochemistry, Baylor College of Medicine, Houston, TX 77030.

Because of the great diversity of $GABA_A$ receptor subunits, selective antibodies are invaluable reagents for examining receptor polypeptide distribution and regulation. Since covalent labeling of these subunits is a useful adjunct to such studies, it is important that the antibodies have high titers for immunoprecipitation. Accordingly, polyclonal antibodies were produced from an intracellular loop region of the chick $GABA_A$ receptor β4 subunit. The β4S(316-437) sequence was expressed as a fusion protein containing a $(His)_6$ leader peptide, purified by Ni^{2+}-affinity chromatography, and used for rabbit immunizations. The resulting RK3-6 antiserum showed a high titer (EC_{50} = 0.1 µl) for immunoprecipitation of ^{3}H-flunitrazepam binding from extracts of chick embryo cortex and reacted with a single 56-57 kDa polypeptide on Western blots. After preabsorption of RK3-6 antiserum with a β2S(317-428) fusion protein, a $1:10^4$ dilution reacted strongly on slot blots with 10 ng of the β4S(316-437) and β4L(316-441) fusion proteins but not with those containing β2S(317-428), β2L(317-445), or α1(331-381) $GABA_A$ receptor subunit sequences. Saturating levels of the preabsorbed β4 antibody immunoprecipitated 66 ± 3% and 38 ± 4.5% of ^{3}H-flunitrazepam binding to extracts from 16-day-old chick embryo and adult chicken cerebrum, respectively. The results suggest that the β4 subunit of the $GABA_A$ receptor represents an embryonic isoform.

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PEPTIDE RECEPTOR STRUCTURE AND FUNCTION IV

724.1

CLONING AND CHARACTERIZATION OF THE RAT GALR1 GALANIN RECEPTOR FROM RIN 14B INSULINOMA CELLS E.M. Parker*, D.G. Izzarelli, H.P. Nowak, C.D. Mahle, L.G. Iben, J. Wang and M.E. Goldstein, Dept. of Psychobiological Disorders, Bristol-Myers Squibb Co., Wallingford, CT

Galanin is a ubiquitous neuropeptide that regulates a wide array of physiological processes via interaction with specific G protein-coupled receptors. A rat galanin receptor cDNA was cloned from the Rin14B insulinoma cell line. The isolated cDNA encodes a 346 amino acid G protein-coupled receptor that is 92% identical to the recently reported human GALR1 galanin receptor. [^{125}I]Galanin binds to high and low affinity states of the rat GALR1 receptor in COS1 cell membranes ($K_{D\text{-}H}$=19 pM, $B_{max\text{-}H}$=0.47 pmol/mg protein, $K_{D\text{-}L}$=300 pM, $B_{max\text{-}L}$=1.63 pmol/mg protein). Galanin, N-terminal galanin fragments and the putative galanin receptor antagonists galantide, C7, M35 and M40 bind with high affinity to the rat GALR1 receptor. In contrast, C-terminal galanin fragments do not bind to this receptor. Galanin inhibits basal and forskolin-stimulated cAMP formation in CHO cells expressing the rat GALR1 receptor via a pertussis toxin-sensitive G protein. The GALR1 receptor is expressed in rat spinal cord, small intestine, Rin14B insulinoma cells and several brain regions, particularly ventral hippocampus, amygdala, supraoptic nucleus, hypothalamus, thalamus, lateral parabrachial nucleus and locus coeruleus. In the late stage rat embryo, the GALR1 receptor is expressed in brain, spinal cord, dorsal root ganglia and small intestine. Cloning of the rat GALR1 galanin receptor cDNA will permit many new experimental strategies to be applied to studies of the structure and function of galanin receptors.

724.2

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE SOMATOSTATIN RECEPTOR SSTR2 IN RAT BRAIN USING A SPECIFIC ANTI-PEPTIDE ANTIBODY. P. Dournaud, Y.Z. Gu‡, A. Schonbrunn*‡, J. Mazella, G.S. Tannenbaum and A. Beaudet. Mc Gill University, Montreal., Québec, Canada, H3A 2B4 and ‡University of Texas, Houston, TX 77225.

In the present study, we have used a rabbit polyclonal antibody generated against a unique sequence in the SSTR2 receptor to visualize the regional and cellular distribution of this receptor subtype in rat brain. Specificity of the antiserum was demonstrated by immunoblot reactivity, and by its selective recognition of cultured COS-7 cells transfected with the cDNA encoding SSTR2, but neither SSTR1 nor SSTR2b receptor subtypes. In rat brain sections, SSTR2-like immunoreactivity was mainly evident within neuronal perikarya and dendrites distributed throughout layers II-III and V of the cerebral cortex, the lateral septum, caudate putamen, nucleus accumbens, central amygdaloid nucleus, medial habenula, locus coeruleus and the pyramidal cell layer of the CA1-CA2 fields of the hippocampus. Dense plexuses of SSTR2-immunoreactive axon-like fibers were also observed in the deep layers of the cortex, claustrum, basolateral nucleus of the amygdala, and superior colliculus. Only sparse, moderately labeled perikarya and fibers were apparent within the hypothalamus, namely in the preoptic area, periventricular nucleus and arcuate nucleus. The thalamus and cerebellum were both devoid of immunoreactivity. In the hippocampus, medial habenula and central amygdaloid nucleus, the immunoreactivity was found by electron microscopy to pervade the cytoplasm of perikarya, dendrites and dendritic spines. In the median eminence, a few axon terminals also displayed reaction product suggesting a presynaptic localization of SSTR2 in this area. The present results provide the first demonstration of the regional and cellular distribution of SSTR2 receptor in rat brain, and suggest that this receptor subtype is involved in the transduction of both pre- and postsynaptic effects of somatostatin in the central nervous system.

724.3

IMMUNOLOGICAL ANALYSIS OF ANGIOTENSIN TYPE 2 RECEPTORS IN THE BRAIN AND PERIPHERY OF DEVELOPING RAT. L.P. Reagan*, S.Y. Chow, L.Y. Ma, D.K. Yee, R.R. Sakai and S.J. Fluharty. Depts. of Animal Biology, Pharmacology, Psychology and the Institute of Neurological Sciences, University of Pennsylvania, PA 19104.

Angiotensin II (AngII) receptor levels are high in neonates and decrease to adult levels as the animal matures. A larger proportion of this reduction is due to decreases in Type 2 (AT_2) receptors rather than Type 1 (AT_1) receptors. In the present study, AT_2 antisera were used to study the distribution of AT_2 receptors in the developing rat brain and in peripheral tissues by immunohistochemical and immunoblot analysis.

Rat brains at 4, 9, and 12 days of age were examined via immunohistochemical techniques and compared with previous results from adult rat brain. As with the mature animal AT_2 receptor immunoreactivity was seen in the locus coeruleus, the supraoptic nucleus and the paraventricular hypothalamic nucleus. AT_2 receptor staining was also associated with the Purkinje cell layer of the cerebellum, as was seen in the adult rat. Dissimilar from the adult, AT_2 receptor immunoreactivity in the neonatal rat pup was less dense in the hippocampus.

Immunoblot analysis was performed to determine the expression of AT_2 receptors in both neonatal and adult rat peripheral tissues. Interestingly, AT_2-directed antisera did not immunodetect proteins in the neonate or adult adrenal, a tissue which is know to exhibit AT_2 binding activity. However, in the neonate a 66 kDa immunoreactive protein was detected in the aorta and the kidney. In neonatal atria, immunoreactive bands of 210, 66 and 50 kDa were observed, with the 66 kDa band being the most intense. In contrast, the major immunoreactive protein was approximately 50 kDa in adult aorta, atria and kidney.

Collectively, these results establish the localization of AT_2 receptors in the developing rat brain, in addition to examining AT_2 receptor expression in neonatal and adult peripheral tissues. These results further support the hypothesis of AT_2 receptor heterogeneity. (Supported by MH43787 and NS23986)

724.4

CLONING AND MUTATIONAL ANALYSIS OF AN ANGIOTENSIN TYPE 2 RECEPTOR FROM MURINE NEUROBLASTOMA N1E-115 CELLS. D.K. Yee*, P. He, X.-D. Yang, L. P. Reagan, L. A. Rogers, J. N. Heerding, J. Hines, and S. J. Fluharty. Depts of Animal Biology, Psychology, Pharmacology, and Institute of Neurological Sciences. Univ. of PA, Phila., PA 19104.

An angiotensin Type 2 (AT_2) receptor identical to other cloned AT_2 receptors has been isolated from a murine neuroblastoma N1E-115 cDNA library using homology-based polymerase chain reaction. When transfected into COS-1 cells, this clone displayed *(i)* AT_2 pharmacology, *(ii)* GTPγS-insensitive agonist binding, and *(iii)* augmentation of agonist binding by dithiothreitol. Previously, we had demonstrated that N1E-115 cells possess two distinct subpopulations of AT_2 receptors, defined as Peak I and Peak III receptors (Siemens et al. *J.Neurochem.* 52:1393, 1994). The pharmacological profile and distinctive ligand binding properties in the presence of GTPγS and dithiothreitol of the cloned AT_2 receptor are consistent with that of Peak III receptors. Moreover, antisera raised against Peak I receptors failed to immunoreact with either Peak III or cloned AT_2 receptors. Collectively, these data suggest that the cloned AT_2 receptor is identical to Peak III receptors and that an apparently novel AT_2 receptor (in Peak I) remains to be cloned.

It was previously shown for the AT_1 receptor that mutating a specific lysine (Lys^{199}) residue to glutamine diminished its affinity for peptidic ligands (Yamano et al., *BBRC* 187:1426, 1992). An analogously mutated AT_2 receptor (Lys^{215}), when expressed in COS-1 cells, possessed greatly reduced affinity for ^{125}I-angiotensin II compared to wild type receptor. More extensive pharmacological analysis of the mutated receptor to both peptidic and non-peptidic ligands is currently in progress. The effects of the lysine mutation on the AT_2 receptor suggests that despite the low homology between AT_1 and AT_2 receptors (only 34%), some commonalities in the binding mechanism for angiotensin II exists between the two receptor subtypes. (Supported by NS23986 and MH43787)

724.5

ANTISENSE OLIGONUCLEOTIDES REDUCE EXPRESSION OF ANGIOTENSIN TYPE 2 RECEPTORS IN DIFFERENTIATED MURINE NEUROBLASTOMA N1E-115 CELLS. L. A. Rogers, D. K. Yee, and S. J. Fluharty*. Depts. of Animal Biology, Pharmacology, Psychology, and Institute of Neurological Sciences. Univ of PA. Phila., PA 19026.

Previously, we have demonstrated that murine neuroblastoma N1E-115 cells possess both Type 1 (AT_1) and Type 2 (AT_2) angiotensin receptors. Inducing differentiation of these cells by the addition of dimethyl sulfoxide (DMSO) results in up to a ten-fold increase in the expression of AT_2 receptors. In order to evaluate the efficacy of antisense oligonucleotides in reducing expression of this angiotensin receptor subtype, either AT_2 antisense or scrambled 15-mer *S*-oligonucleotides were administered concurrently with DMSO treatments. Following three 24 hr treatments of the *S*-oligos, the cells were harvested and AT_2 receptor expression was determined by radioligand binding assays. At concentrations of either 50 *n*M or 500 *n*M, the AT_2 antisense oligoucleotide effectively attenuated the upregulation of these receptors during differentiation by approximately 40% compared to cell cultures treated with scrambled oligonucleotides. These studies are the first demonstration that antisense oligonucleotides decrease *in vitro* the expression of the AT_2 receptor subtype and therefore should prove to be an important new tool in studying function and regulation of this angiotensin receptor subtype. (Supported by NS23986 and MH43787)

724.6

HIGH EXPRESION OF VASOPRESSIN RECEPTORS (AVP-R) IN THE THALAMUS OF GENETICALLY OBESE RATS (fa/fa). Y. Arsenijevic[1], F. Rohner-Jeanrenaud[2], E. Tribollet[1], M. Muhlethaler*[1] and B. Jeanrenaud[2]. [1]Dept. of Physiology, Univ. Med. Center, [2]Lab. Metab. Res., Dept. of Medecine, 1211 Genève 4.

The rat Zucker strain has a recessive mutation responsible for obesity in homozygous animals. Obese fa/fa rats present numerous abnormalities, in particular hyperinsulinemia, hypogonadism and an altered response to stress. In normal rats, AVP-R and oxytocin receptors (OT-R) are present in brain regions implicated in the regulation of these parameters, i.e. the hypothalamus, the hippocampus and the dorsal motor nucleus of the vagus nerve. This suggests 1) that AVP and OT may participate to this regulation and 2) that the expression of AVP-R and OT-R may differ in obese rats. To test these hypothesis, brain slices of obese and lean rats were labelled using two iodinated ligands specific for V_{1a} AVP-R and for OT-R respectively. Autoradiogramms were quantified with an image analyser: the density of the receptors was measured and the number of receptors per labelled structure was estimated. No differences were detected in the hypothalamus, the hippocampus, and the brainstem between both groups, while in the thalamus, AVP-R were much more numerous in obese than in lean rats: the number of AVP-R was 40 % higher in the thalamic ventromedial nucleus, and 100% higher in the ventrolateral, the mediodorsolateral and the posterior thalamic nuclei. These four nuclei are essentially implicated in motor control. The physiopathological significance of these results is under investigation.

724.7

VASOPRESSIN-INDUCED CALCIUM SIGNALING IN CULTURED CORTICAL NEURONS. M.C. Son* and R.D. Brinton. Dept. Molecular Pharmacology & Toxicology, Univ. of Southern California, Pharmaceutical Sci. Ctr., 1985 Zonal Ave., Los Angeles, CA. 90033.

Our early evidence of recognition sites in the cerebral cortex for the neuropeptide vasopressin (Brinton et al. PNAS 1984, Chen et al. *Hippocampal*, 1993) suggested the existence of V1 vasopressin receptors. Based on these findings, we have pursued the signal transduction mechanism of V_1 receptors in cultured cortical neurons using a selective V_1 vasopressin receptor agonist. The dose-response of V_1 agonist-induced accumulation of $[^3H]IP_1$ was concentration dependent and showed a significant linear increase from 250nM (138%±16, $p<0.01$) to 1000nM (189%±16, $p<0.001$). A significant increase in the accumulation of $[^3H]IP_1$ was observed within 20min exposure to V_1 agonist. Peptide specificity indicated that the closely related vasopressin metabolite peptides AVP_{4-8} and AVP_{4-9} also induced significant increases in $[^3H]IP_1$ accumulation as did oxytocin. We further determined whether V1 receptor activation regulated the influx of calcium by conducting $^{45}Ca^{2+}$ uptake analyses. Results of these investigations demonstrated that V_1 agonist (250nM) induced a significant increase in $^{45}Ca^{2+}$ uptake from the extracellular medium within 5 sec. V_1 agonist-induced $^{45}Ca^{2+}$ uptake dose-response analysis exhibited a nonlinear profile. These results demonstrate that V1 vasopressin receptor activation leads to calcium signaling in cortical neurons through the PIP_2 pathway and calcium uptake from the extracellular medium. Future studies will investigate V1 agonist-induced increases in intracellular calcium using calcium fluorometry analysis.

Supported by NIH grant 460366 to R.D.B.

724.8

Vasopressin-Induced Specific Gene Expression in the Cultured Cortical Neurons. Qi Chen*, S. Schreiber and R.D. Brinton. Depart. Molecular Pharmacology & Toxicology, Univ. of Southern California, Pharmaceutical Sciences Center, 1985 Zonal Ave., Los Angeles, CA 90033

Work from our laboratory has demonstrated the existence of V1a vasopressin receptors (V1aR) in cerebral cortex (Brinton et al, 1989, Yamazaki et al, 1995). In addition, we have found V1aR induces calcium signaling in cortical neurons and astrocytes (Son and Brinton, 1995). Downstream effects induced by V1aR activation are, however, still unknown. We have investigated specific gene expression induced by V1aR activation in cultured rat cortical neurons by using differential display PCR. Eighteen-day-old rat cortical neurons were cultured in serum-free media for 16 hours and treated with 250nM V1aR agonist for 15 min, 2 hours and 24 hours, respectively. Total RNAs were isolated and subjected to differential display PCR by using an anchor oligo(dT) primer and an arbitrary primer. Differential patterns were run on the polyacrylamide gels and visualized by silver staining or labelled by ^{35}S-dATP. Our preliminary results indicate V1aR-induced specific gene expression. Further identification by Northern blot analysis and sequencing will be conducted.

supported by NIH grant MH46036 to R.D.B.

724.9

V1a VASOPRESSIN RECEPTOR GENE EXPRESSION IN CULTURED CORTICAL NEURONS, ASTROCYTES AND OLIGODENDROCYTES. R.S.Yamazaki[1]*, Q.Chen[1], S. Schreiber[2] and R.D. Brinton[1]. Dept of Molecular Pharmacology & Toxicology[1], Dept. of Neurology[2], Univ. of Southern California, Pharmaceutical Science Center, 1985 Zonal Ave., Los Angeles, CA 90033

Our early evidence of recognition sites in the cerebral cortex for the neuropeptide vasopressin (Brinton et al. *PNAS* 1984, Chen et al. *Hippocampus*, 1993) suggested the existence of V1 vasopressin receptors. To date there has been no direct evidence of the specific type of cells in cerebral cortex that express the V1 receptor. We pursued this question by using selectively enriched cultures of cortical neurons, astrocytes, and oligodendrocytes. Dissociated cortical cells derived from E18 rat pups were cultured in T-75 flasks in serum containing medium. Neurons were collected after 4 days in vitro (DIV). Oligodendrocytes and astrocytes were collected separately after 21 DIV. Total RNA from each fraction was extracted and purified. To convert mRNA to cDNA and amplify the signal, RT-PCR was performed on each sample using 2 sets of 20mer oligonucleotides targeted to a vasopressin V1a receptor (V1aR) sequence of ~350 bp and a vasopressin V1b receptor (V1bR) sequence of ~500bp. Samples were electrophoresed and immobilized on nylon membranes. Hybridizations of the samples with ^{32}P-labeled probes directed against V1aR and V1bR detected the presence of V1a cDNA, and the absence of V1bR cDNA, in all 3 cell types in the cerebral cortex. These data suggest that distinct vasopressin receptor types are localized in cortex and that within the cortex, the V1a receptor type is expressed in each of the 3 major cell types. Consequently, vasopressin may have pleiotropic effects in the cerebral cortex. In situ hybridization studies to determine the magnitude of expression and proportion of each cell type that expresses V1aR are in progress.

Supported by NIH grant MH46036 to R.D.B.

724.10

IDENTIFICATION OF OPIOID RECEPTOR-LIKE SEQUENCES AMONG DIFFERENT SPECIES. X. Li*, D.E. Keith Jr., C.J. Evans. Department of Psychiatry and Biobehavial Sciences, University of California at Los Angeles School of Medicine, Los Angeles CA 90024-1759

The DNA sequences of members of the opioid receptor family (mu,delta kappa and the orphan ORL-1) have regions highly conserved - a conservation that is retained in human, rat and murine sequences. Areas that are highly homologous within this family of G-protein coupled receptors include the intracellular loops and the transmembrane domains. In order to gain insights into the evolution of the opioid receptor family, degenerate primers were designed according to sequences highly conserved within the family and polymerase chain reaction (PCR) was used to screen genomic DNA from a number of different species. DNA was assayed both from the protostome and deuterostome branches of the metazoan phylogenetic tree. The specific products from PCR were subcloned into pCRII and sequenced. Opioid receptor-like sequences were obtained from beef, chicken, fish and frog. No specific opioid like sequences were obtained from *C-elegans* or *drosophila*. In frog, we obtained three different receptor sequences that were highly homologous but not identical to the mu, delta, and kappa opioid receptors previously isolated from mouse. Among the three receptors identified from frog, the kappa receptor was most divergent from the murine counterpart in the PCR sequence that was analysed. Lower species are presently being investigated to more fully understand the evolution of the opioid receptor family. Supported by NIDA DA05010 and W.M. Keck Foundation.

724.11

A NOVEL BOMBESIN-LIKE-PEPTIDE ISOLATED FROM THE FROG *RANA CATESBEIANA*. B.J. Barry, S.R. Nagalla, M.S. Smith*, E.R. Spindel. Div. Neuroscience, OR Rgl. Primate Research Ctr., Beaverton, OR 97006.

Bombesin was originally characterized in the skin of *Bombina bombina*. Many bombesin-like peptides (BLPs) have since been isolated from various amphibians and mammals. The BLPs are usually divided into three subfamilies; the GRP subfamily, the NMB subfamily, and the bombesin-related peptide (BRP) subfamily which contains bombesin, ranatensin, and phyllolitorin. Traditionally, the ranatensins have been distinguished from the bombesins by the presence of a Phe rather than a Leu as the penultimate C-terminal amino acid; however a Phe form of bombesin has recently been cloned. This discovery indicates that bombesin and ranatensin may be more closely related than thought, while also increasing the apparent complexity of BLPs. Previously Ranatensin C, which is highly homologous to ranatensin, was characterized in *Rana catesbeiana*. Using RT-PCR and degenerate oligos we have identified a new BLP, Ranatensin-V, in *R. catesbeiana* skin. Ranatensin-V has a valine in the penultimate position- the only BLP as yet to have this feature.

Bombesin	**Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met.NH2**
Ranatensin	**pGlu-Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-Met.NH2**
Ranatensin-C	**pGlu-Thr-Pro-Gln-Trp-Ala-Thr-Gly-His-Phe-Met.NH2**
Ranatensin-V	**pGlu-Thr-Pro-Gln-Trp-Ala-Val-Gly-His-Val-Met.NH2**

Binding experiments have indicated that the penultimate amino acid is important for the binding of BLPs to potential receptors. Thus this new peptide may identify an entirely new class of BLPs characterized by a penultimate Val, or alternatively may derive from a conservative evolutionary amino acid switch from Leu to Val. Just as Phe and Leu forms of both bombesin and phyllolitorin have been isolated, this new ranatensin may actually be the Val/Leu form of ranatensin as a complement the previously characterized Phe form.

724.12

IDENTIFICATION OF NOVEL CHEMOKINE-LIKE RECEPTORS IN THE MAMMALIAN CNS BY PCR M.E. Charlton*, S.R. Marsh, A. M. Ciabarra and R.S. Duman. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06598

Over the past several years studies in our laboratory have focused on the function of several discrete brain regions implicated in the etiology and treatment of psychiatric disorders. In order to ultimately study the molecular actions of psychotropic drugs within these brain regions we have initiated studies to identify novel receptors belonging to the G-protein coupled receptor superfamily. Using a PCR based approach with degenerate primers corresponding to regions highly conserved among particular receptor subfamilies, our laboratory has identified several cDNA fragments encoding putative G-protein coupled receptors from discrete rat brain regions including the ventral tegmentum (VT) and locus coeruleus (LC). Sequence analysis of the novel clones VTR 15-20 and LCR 3-12 has revealed a homology at the amino acid levels to the IL-8 and Monocyte Chemoattractant Protein (MCP) receptors, respectively. The distribution pattern and relative expression levels of the mRNA encoding these clones was determined by RNase protection assay and northern blot analysis. The highest expression levels were detected in peripheral tissues (spleen, lung, heart, liver) and lower levels of mRNA expression were apparent in CNS regions (thalamus, pons, cortex, hippocampus, and cerebellum). We are currently isolating full length clones encompassing VTR 15-20 and LCR 3-12 from a rat spleen cDNA library. Further studies will include characterization of the ligand specificity, pharmacology, and the regulatory properties of these receptors. These studies demonstrate the existence of these novel chemokine-like receptors in brain and suggest a role for chemokines in the regulation of brain function.

724.13

Molecular Cloning of a Rat C-C Chemokine Receptor; Expression in Cultured Microglia. J.K. Harrison[1]*, Y. Jiang[1], M.N. Salafranca[1], N.A. Pennell[2], and W.J. Streit[2]. Departments of Pharmacology[1] and Neuroscience[2], University of Florida.

Chemoattractant cytokines (chemokines) are a class of pro-inflammatory peptides that are important mediators of leukocyte migration. While these agents have been well characterized in peripheral systems and models of peripheral inflammation, little is known about the role or function of these cytokines in normal and pathological states of the central nervous system (CNS). Receptors for some of the chemokine peptides have been identified by molecular cloning and are members of the large gene superfamily of G-protein coupled receptors (GCRs). Using degenerate oligonucleotide primers encoding highly conserved regions found in known chemokine receptors, we amplified (by the polymerase chain reaction) DNA fragments encoding several novel members of the GCR superfamily. These fragments were used subsequently to screen rat genomic DNA libraries. Sequence analysis of one of these genomic clones (gCrec13) revealed a translational open reading frame of 354 amino acids. The conceptualized amino acid sequence is 77% identical to the human monocyte chemoattractant protein-1 (MCP-1) receptor, with the greatest divergence in the N-terminus and second extracellular loop. Northern analysis of cultured rat microglia demonstrated the presence of mRNA hybridizing to Crec13 DNA. Treatment of the cultures with Interferon-γ resulted in a dramatic up-regulation of this mRNA. We are hypothesizing that the rat CNS (and specifically microglia) express functional MCP-1 receptors.

724.14

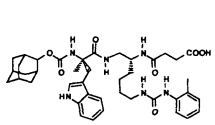
COMPARATIVE INTERNALIZATION PROFILE OF INSULIN-LIKE GROWTH FACTORS (IGF-I and IGF-II) IN A PRIMARY CULTURE MODEL OF RAT HIPPOCAMPAL NEURONS. R. Quirion*, S. Doré, and S. Kar. Douglas Hosp. Res. Ctr, Dept Psychiatry, McGill University, Montreal, Quebec, Canada, H4H 1R3.

IGF-I and IGF-II are structurally related peptides with distinct actions in the brain. Their physiological responses are mediated by specific receptors which are uniquely distributed throughout the brain, being especially concentrated in the hippocampus. We have shown using superfusion of hippocampal slices that concentrations of 10^{-14} to 10^{-8} M of IGF-I inhibit whereas IGF-II potentiates K^+ (25mM)-evoked acetylcholine release (Soc. Neurosc. Abst. 20:81, 1994). After establishing the optimal conditions for the culture of embryonic (E18) rat hippocampal neurons, binding properties of IGF receptors were characterized (Soc. Neurosc. Abst. 20:40, 1994). Competition with insulin revealed the presence of both the type I and type II IGF receptors on these neurons. In order to investigate the putative internalization profile of the IGF-I and IGF-II /receptor complexes in this model, a technique of acid stripping was used. Both total and internalized ^{125}I-IGF-I and ^{125}I IGF-II labelling reached a plateau after a 20 minute incubation at 37°C. The apparent speed of internalization profile was more pronounced for the IGF-II receptor complex than for the IGF-I receptor. The internalization of IGF-I and IGF-II was confirmed by using phenyl arsine oxide (10μM), a general inhibitor of endocytosis, which significantly blocked both internalization processes. In order to eliminate the possible recycling of receptors under our experimental conditions, an inhibitor of receptor-recycling mechanism, monensin (10μM) was used and shown to have no effect on the internalization of either ligands. As the type I IGF receptor is coupled to tyrosine kinase activity, tyrphostin 47 (0.1-10mM), a specific tyrosine kinase inhibitor, was used and showed to significantly decrease IGF-I/receptor internalization without affecting the IGF-II/receptor complex. Hence, the IGF-I is apparently mostly internalized via the type I receptor while IGF-II is not. Taken together, these results provide further evidence for the existence of at least two classes of IGF receptors in the hippocampal formation, the IGF-I ligand receptor complex being internalized via a tyrosine kinase sensitive pathway. In contrast, the IGF-II ligand receptor complex is likely internalized via a different pathway possibly related to a mannose-phosphorylated residues as the IGF-II/mannose-6-phosphate has been implicated in the intracellular targeting of lysosomal proteins containing mannose-6-phosphate residues. (Supported by the MRCC and The Alzheimer Society of Canada).

724.15

DIFFERENT OCCUPANCY OF AGONISTS AT THE CCK-A RECEPTOR R. Ray, K. Doring, H. Schmitthenner, R. Simmons and J. R. Bostwick*.
Dept. Biology, Fisons Pharmaceuticals, 755 Jefferson Rd., Rochester, NY 14623

A recent report that a CCK peptoid analog, Compound *I*, is an agonist prompted us to investigate the nature of its interaction with the CCK-A receptor. We compared the effect of Compound *I* on the binding of an agonist, [^{125}I]CCK8, and an antagonist, [^{3}H]MK329, in rat pancreas membranes. Scatchard analysis confirmed that CCK8 competitively blocked [^{3}H]MK329 binding in this preparation, with 10 nM CCK8 increasing the apparent K_d for [^{3}H]MK329 binding from 0.3 nM to 2 nM with no effect on B_{max}. Compound *I* competitively displaced [^{125}I]CCK8 binding. In the presence of 5 nM Compound *I*, the apparent K_d for [^{125}I]CCK8 shifted from 20 pM to 225 pM with no change in B_{max}. In contrast, 100 nM Compound *I* was a non-competitive inhibitor of [^{3}H]MK329 binding, decreasing B_{max} from 244 pmoles/gram tissue to 78 pmoles/gram tissue. These data show that two compounds, both of which compete with CCK8 for binding at the A receptor, are noncompetitive with each other. The finding that Compound *I* binding is noncompetitive with [^{3}H]MK329 whereas CCK8 binding is competitive with [^{3}H]MK329 indicates that the interaction of each agonist with the CCK-A receptor is different. We propose a model wherein Compound *I* and MK329 occupy separate but allosterically linked domains of the CCK8 binding region in the A receptor.



Compound *I*

724.16

CHARACTERISATION OF RADIOLIGAND BINDING BY SCINTILLATION PROXIMITY ASSAY TO ANGIOTENSIN II TYPE 1, DOPAMINE D_2 AND 5-HYDROXYTRYPTAMINE TYPE 1A RECEPTORS EXPRESSED IN INSECT CELLS USING THE BACULOVIRUS SYSTEM. G. O'Beirne[1], A.J. Harris[1], M. Dennis[2], A. Patel[1]* and N.D. Cook[1]. 1. Amersham International plc, Cardiff Labs., Whitchurch, Cardiff, Wales, U.K. CF4 7YT. 2. BioSignal Inc. 1744 rue William, Montréal, (Québec), Canada. H3J 1R4.

Scintillation Proximity Assay# (SPA) technology was used to characterise the binding of radioligands to various human recombinant receptors expressed in Sf 9 insect cells via the baculovirus system. It was possible to couple the Sf 9 cell membranes containing the baculovirus expressed receptors to both wheatgerm agglutinin (WGA) and polylysine coated SPA beads. The SPA beads contain scintillant which detects low energy emissions (ie: β-particles from ^{3}H or Auger electrons from ^{125}I) only when the radioisotope is in close proximity to the bead. Consequently, in SPA receptor binding assays it is not necessary to separate receptor-bound ligand from free, as only radioligand bound to receptors coupled to the beads causes light to be emitted.

In these experiments the binding characteristics of transfected receptors were determined by SPA using WGA SPA beads and compared to those determined using traditional filtration methods. Three different receptor/ligand systems were examined in detail; [^{125}I][Sar^{1}Ile^8]angiotensin II binding to angiotensin II type 1 receptors, [^{125}I]epidepride binding to dopamine D_2 receptors and 8-hydroxy-[^{3}H] dipropylaminotetralin (8-OH-[^{3}H]DPAT) binding to 5-hydroxytryptamine type 1A ($5HT_{1A}$) receptors. Comparable K_D values were found when saturation binding experiments were performed by both SPA and filtration methodologies. These studies demonstrate that it is possible to use SPA to characterise binding to recombinant receptors expressed using the baculovirus insect cell system.

Scintillation Proximity Assay technology is covered by US Patent No. 4568649, European Patent No. 0154734 and by Japanese Application No. 84/52452.

724.17

DESIGN OF NEW CRF ANALOGUES AND MOLECULAR CHARACTERIZATION OF FUNCTIONAL CRF RECEPTORS. Andreas Rühmann, Frank M. Dautzenberg, Andreas K. E. Köpke and J. Spiess*. Max-Planck-Institute for Exp. Med. Dept. Mol. Neuroendocrinology, Hermann-Rein-Str. 3, D-37075 Goettingen, Germany.

Corticotropin releasing factor (CRF) is an early signal in stress response. A functional CRF receptor which increases adenylate cyclase activity on stimulation by CRF has been demonstrated in the human Y79 retinoblastoma line [Olianas, M.C., Lampis, G., and Onali, P., 1995, J. Neurochem. 64, pp 402].

On these findings, newly designed lipophilic CRF agonists and antagonists have been developed which show similar binding affinities and biopotencies when compared to h/rCRF (1-41) and the antagonist [DPhe^{12}, Nle^{21,38}] h/rCRF (12-41), respectively.

In a PCR based approach we could demonstrate that CRF_1 receptor is expressed in Y79 cells. Furthermore, three different variants of this receptor type were found. The first variant encodes the previously cloned CRF_{1a} receptor polypeptide. The two additional variants are novel. Both have deletions of different lengths in the extracellular N-terminal domain. The presence of different receptors is consistent with our binding data performed on Y79 membrane homogenates, that favor the existence of high- and low-affinity binding sites.

For the identification of the ligand binding site with a potent photoactivatable CRF analogue, HEK 293 cells were transfected with the rat CRF_1 receptor. The CRF binding constant did not deviate significantly from the corresponding values of pituitary or Y79 cell receptors. However, the CRF receptor density was significantly increased in HEK cells when compared to the receptor density in Y79 cells. The ongoing production of stable cell lines expressing functional CRF receptors at higher level will facilitate the structure activity relationship (SAR) studies with CRF agonists and antagonists.

724.18

IRREVERSIBLE ANTAGONISM OF THE GONADOTROPIN-RELEASING HORMONE RECEPTOR TO EVALUATE " SPARE RECEPTORS" IN TRANSIENT TRANSFECTION SYSTEMS. S. Kitanovic*, S.C. Sealfon. Fishberg Center for Neurobiology and Dept. of Neurology, Mount Sinai Medical Center, New York, NY 10029

Transient transfection systems are frequently employed to study the coupling of wild type (WT) and mutant receptors. However, EC_{50} values obtained in functional studies may depend on the level of receptor expression per cell. An approach sometimes used to determine the relationship between EC_{50} and receptor expression following transient transfection is to alter the amount of receptor DNA being transfected into cells. However, the interpretation of results obtained is not conclusive because it is not known whether decreasing the amount of transfected plasmid causes a change in the expression of receptors per cell or in the number of cells expressing the receptor. We have evaluated the relationship between measured EC_{50} and the concentration of the gonadotropin-releasing hormone receptors (GnRHR) in COS-1 cells by partial irreversible antagonism. Functional receptors were progressively eliminated by pretreating cells with 2,4,6-trinitrobenzenesulfonic acid (TNBS). With the greatest reduction in receptor concentration, EC_{50} values obtained from concentration-response curves increased no more than 3-5 fold. In addition, a decrease in the maximal response was observed. Using the analysis developed by Furchgott (Advances in drug research,3,21-55), the KA of the WT human GnRHR was determined to be approximately 4 nM. These data indicate that the presence of spare receptors can be accurately evaluated in a transient transfection system and therefore this system can be utilized to determine agonist affinity for mutated receptors via the functional response. (Supported by NIH R01 DK-46943 and G3-5615 training grant)

724.19

NOVEL ACTIVATION AND INTERACTION OF SYMPATHETIC NEURON SIGNAL TRANSDUCTION PATHWAYS BY PITUITARY ADENYLATE CYCLASE POLYPEPTIDES. K. M. Braas* and V. May. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Pituitary adenylate cyclase activating polypeptides (PACAP-27 and -38) belong to the VIP/secretin/glucagon family of bioactive peptides and have diverse neuroendocrine regulatory effects. The PACAP-selective type I receptor is positively coupled to adenylate cyclase and phospholipase C (PLC); splice variants of the type I PACAP receptor differentially stimulate adenylyl cyclase and PLC. In the current studies, cultured superior cervical ganglion (SCG) neurons, which expressed predominantly the HOP cassette-containing type I PACAP receptor isoform, displayed potent and efficacious stimulation of catecholamine and NPY secretion by PACAP peptides. Unlike previously studied tissues, SCG inositol phosphate and cyclic AMP production were potently stimulated by both PACAP27 and PACAP38. PACAP38 was more potent than PACAP27 in stimulating SCG cyclic AMP levels; PACAP38 exhibited a half-maximal effect at 0.4 nM compared to 4.0 nM for PACAP27. PACAP27, however, was more efficacious than PACAP38. In contrast to other tissues, PACAP27 and PACAP38 were nearly equipotent in stimulating inositol phosphate production exhibiting a half-maximal response at approximately 0.5 nM. PACAP27- or PACAP38-elicited interactions of these two signaling pathways were investigated using the PLC inhibitor U73122 and/or the adenylyl cyclase inhibitor SQ22536. U73122 attenuated both PACAP27- and PACAP38-stimulated inositol phosphate production, while SQ22536 had no effect. In contrast, PLC inhibition potentiated PACAP27- and PACAP38-stimulated cyclic AMP production. These results indicate that the concerted interactions of these two signal transduction pathways contribute to SCG responses to PACAP. Supported by HD27468 and NS01636 (VM) and AHA94015540 (KMB).

724.20

CHARACTERIZATION OF CALCITONIN RECEPTORS EXPRESSED IN DIFFERENTIATED IMR 32 HUMAN NEUROBLASTOMA CELLS. G. Campana, L.Carboni, M. Canossa E. Speroni, S. Ferri and S.Spampinato*. Dept. Pharmacol., Univ. Bologna,I40126 Bologna, Italy.

Neuroblastoma-derived cell lines express different neuropetide receptors and represent an useful model to investigate their molecular functions in differentiated cells which acquire several phenotypes of mature neuronal cells. We have examined a series of human neuroblastoma cell lines with the aim to detect, by polymerase chain reaction, any mRNA coding for the calcitonin receptor by using two primers complementary to an unique sequence of a cDNA described by Kuestner et al. (Mol.Pharmacol.46,246,1994). Electophoretic analysis of reverse-transcribed total RNA, provided evidence that mRNA coding for this receptor occurs in IMR 32 cells. In these cells, differentiated with 1 mM dibutyril cAMP for 12-14 days and loaded with fura-2, human calcitonin (IC_{50}= 10^{-8}M) and salmon calcitonin (IC_{50}=10^{-9}M) antagonized Ca^{2+}entry evoked by high extracellular K^+(50 mM) or ATP (1 mM). This response was not observed following removal of extracellular Ca^{2+} or in the presence of ω-conotoxin GVIA (20 nM).Therefore, differentiated IMR 32 cells represent an useful tool to study the gene expression and molecular interactions of human calcitonin receptor in neuronal cells.

NEUROTRANSMITTER PROCESSING

725.1

***APLYSIA* CARBOXYPEPTIDASE E: MOLECULAR CLONING, STRUCTURE, AND CELLULAR LOCALIZATION.** G.T. Nagle*[1], W.R.A. van Heumen[2], R. Rodriguez[1], and X. Fan[1]. [1]Marine Biomedical Institute and Department of Anatomy & Neurosciences, University of Texas Medical Branch, Galveston, TX 77555 and [2]Vision, Touch and Hearing Research Centre, University of Queensland, Brisbane, Australia.

The abdominal ganglion neuroendocrine bag cells of *Aplysia* express the egg-laying hormone (ELH) gene and process the resulting polyprotein ELH precursor at multiple sites. Carboxypeptidase E (CPE)-like removal of C-terminal basic amino acids has been previously detected in neural tissue of *Aplysia*. To characterize this processing enzyme, an abdominal ganglion cDNA library was constructed and screened using a CPE-related PCR product generated from the bag cells. The longest clone that was isolated contained the entire open reading frame and encoded a preproenzyme that was most closely related to vertebrate CPE sequences. The cellular localization of *Aplysia* CPE is currently being examined by *in situ* hybridization. The C-terminal region of vertebrate CPE contains the membrane anchor and may contribute to the sorting of this protein into the regulated pathway. Interestingly, the C-terminal region of *Aplysia* CPE showed a low sequence identity with the C-terminal region of vertebrate CPEs.

725.2

***APLYSIA* PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE: MOLECULAR CLONING, STRUCTURE, AND CELLULAR LOCALIZATION.** X. Fan[1], W.R.A. van Heumen[2], S.D. Painter*[1], R. Rodriguez[1], H. Shen[1] and G.T. Nagle[1]. [1]Marine Biomedical Institute and Department of Anatomy & Neurosciences, University of Texas Medical Branch, Galveston, TX 77555 and [2]Vision, Touch and Hearing Research Centre, University of Queensland, Brisbane, Australia.

The abdominal ganglion neuroendocrine bag cells of *Aplysia* express the egg-laying hormone (ELH) gene and process the ELH precursor to generate amidated ELH and other peptides. In addition, these cells and other abdominal ganglion neurons express a homolog of neuropeptide Y that is also amidated. To characterize *Aplysia* peptidylglycine α-amidating monooxygenase (PAM), an abdominal ganglion cDNA library was constructed and screened using a PAM-related PCR product generated from the bag cells. The clone that was isolated encoded a PAM-related enzyme that was most closely related to vertebrate PAM sequences. The cellular localization of *Aplysia* PAM is currently being examined by *in situ* hybridization.

725.3

SUBTILISIN LIKE ENZYMES PC2, PC3/PC1 AND FURIN IN UNDIFFERENTIATED AND DIFFERENTIATED HL-60 CELLS. Osvaldo Vindrola[a]*, Maria Claudia Kleid[b] and Maria Rosa Padros[a]. [a]Instituto de Fisiologia. Universidad Autonoma de Puebla. Puebla. Mexico. [b]Instituto de Investigaciones Medicas. Facultad de Medicina. Universidad de Buenos Aires. Buenos Aires. Argentina.

Prohormone converting enzymes PC2 and PC3/PC1 (PC3) have been detected in rat neutrophils and alveolar macrophages, respectively. In order to ascertain in which stage of the myeloid differentiation processes these enzymes are produced, we studied PC2, PC3 and furin content in undifferentiated (UD) and differentiated (D) human promyelocytic leukemic cell line HL-60. PC2, PC3 and furin were assayed by gel electrophoresis and immunoblotting in UD-HL-60 cells, in neutrophil-like (NL) and macrophage-like (ML) D-HL-60 cells. 68 kDa PC2 protein was detected in UD-HL-60 cells and its levels decreased dramatically in NL and ML D-HL-60 cells. Comparative low concentrations were observed in NL D-HL-60 cells and peripheral human neutrophils. Furthermore, a decrease of PC2 content occurred in peripheral rat neutrophils when compared with enzyme levels in rat bone marrow neutrophils. High concentrations of 66 kDa PC1 protein were detected in UD- and D-HL-60 cells, reaching the highest values in ML D-HL-60 cells. PC3 did not appear in human peripheral neutrophils. 97 kDa furin was not found in HL-60 cells nor in human peripheral neutrophils. However, furin antibody recognized the 66 kDa PC3 protein, reproducing the results obtained with PC3 antibody. HL-60 is a regulated non-endocrine and non-nervous cell, and contained high levels of PC2 and PC3 enzymes, with the absence of furin. These results suggest that PC2 enzyme may be involved in the processing of proproteins in early stages of HL-60 differentiation processes, while PC3 may participate in early as well as in late stages of HL-60 differentiation, specially in ML cells.

725.4

PURIFICATION AND CHARACTERIZATION OF RECOMBINANT PC2. N.S. Lamango and I. Lindberg*, Dept. of Biochemistry and Molecular Biology, Louisiana State University School of Medicine, New Orleans, LA 71002

Following the finding that the neuroendocrine polypeptide 7B2 is necessary for the biosynthesis of enzymatically active prohormone convertase 2 (PC2) (Zhu and Lindberg, J. Cell Biol., *in press*) we have obtained large quantities of the recombinant enzyme from the conditioned medium of PC2-producing CHO cells supertransfected with cDNA coding for 21 kDa 7B2. We have purified the recombinant enzyme to apparent homogeneity with a 40 % recovery. Three protein bands of M_rs 66, 71 and 75 kDa were observed after SDS-PAGE followed by either Coomassie staining or Western blotting with PC2 antiserum. Spontaneous conversion of the 71 and 75 kDa species to the 66 kDa form was observed during incubation; the degree of conversion correlated with increased activity.

Active site titration with the specific PC2 inhibitor $h7B2_{155\text{-}185}$ showed that 27 % of the total protein was active enzyme. K_ms of 124 μM and 131 μM and Kcats of 0.49 ± 0.01 S^{-1} and 0.81 ± 0.02 S^{-1} for the substrates Cbz-Arg-Ser-Lys-Arg-AMC and Pyr-Arg-Thr-Lys-Arg-AMC were observed. The pH optimum of recombinant PC2 was 5.0, and the enzyme was inhibited by $h7B2_{155\text{-}185}$, ρ-CMS and EDTA but not by 1,10-phenanthroline, leupeptin, pepstatin, TPCK, TLCK, E-64 or soybean trypsin inhibitor. Rat proenkephalin was cleaved at multiple sites by the enzyme, suggesting that recombinant PC2 will prove to be a useful tool in the investigation of prohormone maturation.

725.5

EXPRESSION OF PC1 OR PC2 ANTISENSE IN STABLY TRANSFECTED STC-1 CELLS INHIBITS PROCESSING OF PRO CCK. J. Y. Yoon* and M. C. Beinfeld. Dept. Pharm. Physiol. Sci., St. Louis Univ. Med. Ctr., St. Louis, MO 63104 and Dept. Pharm. Exp. Ther., Tufts Univ. Sch. Med., Boston, MA 02111.

The predominant form of cholecystokinin (CCK) in the brain is CCK 8, the second most abundant neuropeptide next to NPY. CCK 8 is an eight amino acid peptide produced from a larger pro CCK precursor and has been implicated in a number of physiological effects including satiety, anxiety, memory and analgesia. A number of posttranslational modifying enzymes are involved in the biosynthesis of bioactive CCK 8 including tyrosine sulfotransferases, endoproteases and amidating enzymes; the identification of many of these enzymes are still under investigation.

Candidate endoproteases include PC1 and PC2, subtilisin like enzymes of the furin family that cleave peptides at monobasic or dibasic residues. Evidence suggesting roles for PC1 and PC2 in pro CCK processing include similar tissue distribution with CCK, both found in endocrine and neuronal tissue, and the expression of both PC1 and PC2 in several tissue culture cell lines that have the ability to processes pro CCK to CCK 8.

In order to further evaluate the role of PC1 and PC2 in CCK processing we have inhibited PC1 or PC2 by constitutively expressing antisense message in the murine intestinal tumor cell line STC-1, which produces not only CCK 8 but also CCK 22, the predominant form of CCK found in rat intestine. We have identified lines that show expression of antisense message by Northern hybridization and significantly reduced levels of PC1 or PC2 protein levels by Western analysis. By radioimmunoassay of G-50 chromatography fractions, preliminary data suggests that the inhibition of PC1 in STC-1 cells specifically inhibits the production of CCK 8 and inhibition of PC2 in STC-1 cells specifically inhibits the production of CCK 22. These data suggest the possible role of both PC1 and PC2 endoproteases in the processing of pro CCK. Supported by NIH grant NS 31602.

725.6

DEVELOPMENTAL EXPRESSION OF PROPROTEIN PROCESSING ENZYMES AND THE PC2 REGULATOR 7B2. M. Zheng[1]*, R. Day[2], N. G. Seidah[2], and J. E. Pintar[3]. [1]Dept. of Anat. & Cell Biol., Columbia Univ. P&S, New York, NY 10032; [2]Lab. of Biochem. Neuroendocrinol., Clin. Res. Inst. of Montreal, Montreal, Quebec H2W 1R7, Canada; [3]Dept. of Neurosci. & Cell Biol., UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

Many peptides modulating cellular growth and differentiation in development are first synthesized as precursors that require proteolytic processing by the "prohormone convertase" (PC) family of endoproteases. Previously we have presented the developmental expression profiles of PC1, PC2, and furin, and compared their patterns to those of potential substrates. We have since compared the expression of PC2 and its regulator 7B2 in development. Expression of these two genes often overlaps but 7B2 expression is dramatically reduced, if not absent, at e17 in multiple CNS regions where PC2 expression is high, such as the dorsal thalamus, hippocampus, and striatum. These differences could result in significant differential stoichiometry of 7B2 and PC2 proteins in these regions. We have also determined that two recently identified PC members, PC5 and PACE4, are also expressed prenatally. PC5 is expressed in the floor plate at e10 and, by midgestation, a low to middle level of PC5 is expressed in several regions including hypothalamus, brain stem, and spinal cord. In contrast, PACE4 expression is detected at high levels in hippocampus and low levels in the thalamus, brain stem, choroid plexus, and spinal cord. In several peripheral organ systems, including lung and gut, we observed remarkably complimentary patterns of PC5 and PACE4 gene expression. In the heart and liver, PACE4 transcripts are expressed (as is furin) at mid- to high levels, whereas PC5 is not expressed to any significant level in these organs. Taken together, these results suggest that both PC5 and PACE4 may be involved in neuropeptide precursor processing in the developing nervous system; the general nonoverlapping expression patterns in peripheral tissues suggest that PC5 and PACE4 may process distinct sets of proprotein substrates. Supported by DA-08622 (JEP).

725.7

MOLLUSCAN PROHORMONE CONVERTASES: STRUCTURAL DIVERSITY IN THE CENTRAL NERVOUS SYSTEM OF *LYMNAEA STAGNALIS* A.B. Smit*, S. Spijker, J. Klumperman and W.P.M. Geraerts. Graduate School of Neurosciences Amsterdam, Institute of Neurosciences, Faculty of Biology, Vrije Universiteit, 1081 HV Amsterdam, The Netherlands

In the central nervous system (CNS) of the mollusc *Lymnaea stagnalis* various prohormones have been characterized, from which (sets of) different neuropeptides can be generated by proteolytic cleavage and posttranslational modifications.

In order to address the enzymes involved in the processing of neuropeptide prohormones in the CNS, we used a PCR and cDNA cloning strategy, and characterized cDNAs corresponding to three different putative endoproteases. One cDNA encodes a protein of 653 residues with an overall sequence identity of ~80% with the catalytic domain of vertebrate PC2, and was called *Lymnaea* PC2 (LPC2). Expression of the LPC2 gene was exclusively found in neurons of the CNS, and two LPC2 transcripts of 3.0 and 4.8 kb were detected. Two other cDNAs were cloned using PCR and furin specific primers and were tentatively called Lfur1 (968 amino acids) and Lfur2 (837 amino acids). Lfur1 shows highest sequence identity to human furin (72%) in the catalytic domain and also the C-terminal region is highly conserved. Lfur2 is structurally related to furin and shows ~70% homology in the catalytic domain with PACE4 and PC5/6, whereas the sequence conservation in the C-terminal part of the protein is low. The Lfur1 gene is expressed in a broad range of tissues, and several sizes of transcripts can be detected. Expression of the Lfur2 gene is also found in many tissues and shows a single transcript of ~6.0 kb. Immunocytochemistry has indicated that the various *Lymnaea* convertases show cell-specific expression, that can be related to the maturation of specific prohormone substrates.

725.8

SECOND MESSENGER EFFECTS ON PROHORMONE CONVERTASES IN THE MEDULLARY THYROID CARCINOMA CELL LINE WE 4/2. B.L. Mania -Farnell*, I. Botros, R. Day, N.G. Seidah and T.P. Davis. Dept. of Bio. Sci., Purdue Univ. Calumet, Hammond, IN 46323; Clinical Res. Institute of Montreal, Montreal, Quebec H2W 1R7; Dept. of Pharmacology, Univ of Arizona, Tucson, AZ 85724.

Regulation of prohormone convertase mRNA expression was studied in the neuropeptide producing cell line WE 4/2, a rat medullary thyroid carcinoma line. To examine the effect of the cyclic AMP (cAMP) second messenger pathway on prohormone convertase 1 (PC1), PC2 and furin mRNA levels, WE 4/2 cells were treated with the phosphodiesterase inhibitor isobutyl-methylxanthine (IBMX, 0.5 mM). Phorbol-12-myristate-13-acetate (PMA, 0.5 uM) was used to determine if the protein kinase C (PKC) pathway regulated prohormone convertase mRNA levels. Messenger RNA levels were quantitated using Northern blot analysis in combination with cRNA hybridization probes, rat (r) PC1, rPC2 and rfurin. In this cell line activation of the cAMP pathway increased mRNA levels of all three prohormone convertases. PC1 mRNA levels increased 1.4 -fold after 3 hour treatments and 1.5 -fold after 6 hour treatments. PC2 mRNA levels increased 1.6 -fold and 1.8 -fold and furin mRNA levels increased 1.5 -fold and 1.8-fold, after 3 and 6 hours respectively. Treatment with PMA or PMA combined with IBMX did not produce significant changes in PC1, PC2 or furin mRNA levels. This pattern of regulation differs from one seen previously in the human neuroepithelioma cell line SK-N-MCIXC (Soc. Neurosci. Abs. 19, 1993), suggesting that prohormone convertases may be differentially regulated by cAMP and PKC mechanisms in a tissue specific manner. Supported by N.I.M.H. # MH42600.

725.9

PRODUCTION OF RAT PRO CCK IN INSECT CELLS INFECTED WITH A RECOMBINANT *BACULOVIRUS*. Margery C. Beinfeld* and Liza Yum. Dept. Pharm. Exp. Ther., Tufts Univ. Sch. Med., Boston, MA. 02111 and Dept. Pharm. Physiol. Sci., St. Louis Univ. Med. Ctr., St. Louis, MO. 63104.

Previous unsuccessful attempts to produce recombinant rat pro CCK in bacteria led us to the *baculovirus* expression system. The rat CCK cDNA was inserted into a transfer vector and was transfected with linearized wild type baculovirus DNA into Sf9 cells. Live virus particles were recovered and amplified by multiple rounds of infection. Production of pro CCK was monitored with the V9M antiserum which detects the amino terminal of pro CCK. Recombinant virus infected Sf9 cells produced a protein of about 10,000 molecular weight and about 80% of this material was secreted, indicating that insect cells recognize the rat signal and sorting sequences. At least half of it appears to be intact prohormone, an additional smaller protein is recovered which appears to be the amino terminal of pro CCK_4. This peptide is a normal cellular product of rat pro CCK processing in rat brain and CCK secreting cell lines which we have also shown to be secreted. Several of our antisera directed against different regions of the pro CCK detects this material, while the CCK 8 antiserum does not. Sf9 cells secrete at least 10 ng/ml while High 5 cells secrete about 5 times more. Secretion is maximal 3 days after infection. Pro CCK could be biosynthetically labeled with ^{35}S sulfate indicating that insect cells are able to sulfate tyrosine residues like other eukaryotic cells. In summary, the *baculovirus* expression system produces significant quantities of intact rat pro CCK for enzymatic and structural studies. Supported by NIH grant NS31602.

725.10

INHIBITION OF DIPEPTIDYL PEPTIDASE IV (CD 26) AND PROLYL OLIGOPEPTIDASE BY PEPTIDYL NITRILES. S. Wilk*, J. Li and E. Wilk Dept. of Pharmacology, Mount Sinai Sch. Med. of CUNY, New York, N.Y. 10029

Dipeptidyl peptidase IV (DPP IV; E.C. 3.4.14.5) and prolyl oligopeptidase (EC 3.4.21.26) are serine proteinases which cleave peptide bonds after proline residues. DPP IV, a membrane-bound ectoenzyme, cleaves dipeptides from the amino terminus of peptides which contain proline as the penultimate amino acid. Substrates include substance P and neuropeptide Y. DPP IV is also identical to the T-cell activation antigen CD 26. Prolyl oligopeptidase, a cytosolic enzyme, cleaves peptidyl prolyl peptide and peptidyl prolyl amino acid bonds. Substrates include substance P, bradykinin, neurotensin, TRH, LHRH and angiotensin. Peptidyl nitriles generally believed to be weak inhibitors of serine proteinases, were found to potently inhibit these two enzymes. DPP IV is inhibited competitively by aminoacyl prolyl nitriles such as Ala-ProCN (Ki=0.2uM). Prolyl oligopeptidase is inhibited non-competitively by Fmoc-aminoacyl prolyl nitriles. The most potent inhibitors were FmocPro-ProCN and FmocAla-ProCN (Ki=5nM). These nitriles are readily synthesized and are stable. They should be of value in studies probing the physiological significance of these enzymes. Supported by NS-17392 and a Research Scientist Award MH-00350 to S.W.

725.11

EXPRESSION OF PROENKEPHALIN (PE) AND IN VITRO PROCESSING OF PURIFIED PE BY THE 'PROHORMONE THIOL PROTEASE' (PTP). V.Y.H. Hook*, M.R. Schiller, and L. Mende-Mueller+. Dept. of Medicine, Univ. of Calif., San Diego, #Dept. of Biochem., Uniformed Services Univ., Bethesda, MD., and +Dept. of Biochem., Med. College of Wisconsin, WI.

The 'prohormone thiol protease' (PTP) is a novel cysteine protease involved in proenkephalin processing. This study examined PTP processing of proenkephalin (PE) to intermediate products, and assessed kinetics of PE processing. Recombinant PE was obtained by high level expression in *E. coli*; PE was then purified by DEAE-Sepharose, gel electrophoresis, and reverse-phase HPLC. Authentic purified PE was confirmed by amino acid composition analyses and peptide microsequencing. In time course studies, PTP converted PE (12 μM) to intermediates of 22.5, 21.7, 12.5, and 11.0 kDa that represented NH_2-terminal fragments of PE, as assessed by peptide microsequencing. Differences in M_r's of the 22.5, 21.7, 12.5, and 11.0 kDa products reflect PTP processing of PE within the COOH-terminal region of PE, which resembles PE processing in vivo. Products of 12.5, 11.0, and 8.5 kDa resulted from PTP cleavage between Lys-Arg at the COOH-terminus of (Met)enkephalin-Arg^6-Gly^7-Leu^8. PTP has a $K_{m(app)}$ value of 18.6 μM PE and $V_{max(app)}$ of 1.98 mmol/hr/mg. These kinetic constants are consistent with intragranular levels of PE and PE-products. Results demonstrating PTP conversion of PE to intermediates resembling those in vivo, and kinetics that are compatible with in vivo PE processing, implicate a role for PTP in PE processing.

725.12

INITIAL PROCESSING OF PROENKEPHALIN IN CHROMAFFIN CELLS OCCURS AT FOUR DIBASIC SITES. S.P. Wilson*, F. Liu and P.R. Housley. Dept. of Pharmacology, University of South Carolina School of Medicine, Columbia, SC 29208.

Processing of proenkephalin was examined in bovine chromaffin cells using recombinant plasmids containing the human prepro-enkephalin (hPPE) cDNA under the control of the cytomegalovirus immediate early enhancer/promoter. Following transfection of hPPE-containing plasmids into the cells, several immunoreactive bands were observed on Western blots with monoclonal antibodies that recognize human, but not bovine, proenkephalin sequences. The pattern of immunoreactive peptides observed was similar to that of endogenous proenkephalin processing products. A series of recombinant plasmids containing mutations in the hPPE sequence at the twelve dibasic processing sites were then constructed. Conversion of Lys-Lys and Lys-Arg sequences to Lys-Gln and of Arg-Arg to Arg-Gln did not alter initial hPPE processing at eight of the twelve putative processing sites. Mutations at the remaining four sites altered the pattern of processing products consistent with blockade of processing at the individual sites. When hPPE cDNA containing mutations at all four of the initially processed sites was expressed none of the normal processing products were found, and a single, novel peptide was produced. These data confirm the importance of dibasic amino acid sequences in proenkephalin processing, but also suggest the importance of secondary or tertiary precursor structure in cleavage of these sites. (Supported by NSF Grant IBN 94-09201)

725.13

PROENKEPHALIN PROCESSING IN NEUROENDOCRINE CELLS: CONTRIBUTION OF PC2. K. Johanning, J.P. Mathis, J. Lyles* and I. Lindberg. Dept. of Biochemistry and Molecular Biology, Louisiana State University School of Medicine, New Orleans, LA 70112.

The biosynthetic pathway for proenkephalin was examined in primary cultures of bovine chromaffin cells, in the rat insulinoma cell line Rin5f stably transfected with rat proenkephalin cDNA (Rin/PE) and in a mouse anterior pituitary tumor cell line, AtT-20 (AT) transfected with either rat proenkephalin (AT/PE) alone or with both pro-enkephalin and mouse prohormone convertase 2 (AT/PC2/PE). In chromaffin cells, pulse-chase experiments showed that within 2 h, proenkephalin was mostly processed to Peptide B, at a rate similar to that previuosly observed in transfected AtT-20 cells. Initial processing of proenkephalin in Rin/PE proceeded much more slowly; full conversion of proenkephalin to Peptide B required at least 12 h. Cleavage of the Peptide B fragment to free Met-enkephalin-Arg-Phe occurred within 6 h in chromaffin cells, more slowly than the identical cleavage event in AT cells (4h). In AT/PC2/PE, this cleavage was complete at 2 h.

RIAs of cellular extracts of Rin/PE cells indicated a a more complete processing profile as compared to AT/PE cells and to chromaffin cells. AT/PC2/PE cells exhibited a similar processing profile as that of Rin/PE cells. These results indicate that PC2 is responsible for the later events of proenkephalin processing.

725.14

HALOPERIDOL DECREASES MET-ENKEPHALIN DEGRADATION AND METABOLITE ACCUMULATION ON REGIONAL BRAIN SLICES. C.S. Konkoy*, S.M. Waters and T.P. Davis. Dept. Pharmacology, Univ. of Arizona, Tucson, AZ 85724.

Previous work in our laboratory provided evidence for regional specificity in the metabolism of met-enkephalin (ME) by intact brain slices. For example, studies with selective peptidase inhibitors suggested that aminopeptidase activity represents the predominant pathway for ME degradation in frontal cortex (FC) whereas other proteases, in addition to aminopeptidases, participate in the degradation of ME in caudate-putamen (CP). Also, subchronic administration (7 day, i.p.) of neuroleptics alters the metabolism of neuropeptides (sub P, CCK and ME) by regional brain slices (JPET, in press). In the present study, selective inhibition of aminopeptidase activity by bestatin (50 μM) decreased the recovery of ME fragments Tyr and Gly-Gly-Phe-Met on FC and CP slices. We inhibited neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE) by phosphoramidon (50 μM) and captopril (50 μM) respectively, thereby decreasing recovery of ME fragment Tyr-Gly-Gly on CP slices. Tyr-Gly-Gly accumulation was sparse in FC, which is consistent with low levels of NEP and ACE activity in this region. Subchronic, but not acute, administration of haloperidol (1 mg/kg) decreased ME degradation on FC (16% vs. control) and on CP (10% vs. control) slices. Haloperidol treatment also decreased accumulation of aminopeptidase-derived fragments Tyr and Gly-Gly-Phe-Met in both brain regions. The accumulation of Tyr-Gly-Gly, however, was not altered by haloperidol treatment in either brain region. These data suggest that the haloperidol-induced decrease in ME degradation results from a selective reduction in the activity of aminopeptidase(s). (Supported by NIMH grant MH42600)

725.15

EFFECT OF DOPAMINERGIC DRUG ADMINISTRATION ON SUBSTANCE P DEGRADATION BY REGIONAL RAT BRAIN SLICES. S.M. Waters*, C.S. Konkoy and T.P. Davis. Dept. Pharmacology, Univ. Arizona, Tucson, AZ 85724.

The neuropeptide substance P (SP) interacts with nigrostriatal and mesocorticolimbic neurons to modulate dopamine (DA) release. Neuroleptic drugs have been shown to decrease the level and mRNA of SP following subchronic administration. Additionally, our laboratory has shown that the metabolism of SP is altered in a regionally specific manner following 7 day administration of the typical neuroleptics haloperidol (1mg/kg) and chlorpromazine (20mg/kg) suggesting altered neuropeptidase activity (J. Pharmacol. Exp. Ther., in press). In the present study, the effect of subchronic (7 day, i.p.) administration of atypical antipsychotics and selective dopamine receptor agonists and antagonists on SP degradation was determined using intact, rat frontal cortex (FC), caudate-putamen (CP) and nucleus accumbens (NA) slices.

In contrast to the decrease in SP degradation in CP and NA following typical neuroleptics, the nonselective DA agonist apomorphine (5mg/kg, bid) significantly increased SP metabolism in NA while the atypical antipsychotic clozapine (20mg/kg) had no effect on SP metabolism in any region studied. Selective DA receptor compounds were used to determine through which receptor subtype haloperidol, chlorpromazine and apomorphine produce their effect on SP metabolism. Neither the selective DA_1 agonist SKF 38393 (1 or 5mg/kg, bid) or the DA_1 antagonist SCH 23390 (0.5 or 2.5 mg/kg, bid) had an effect on SP degradation. Interestingly, the DA_2 antagonist sulpiride (100 mg/kg, bid) was also without significant effect. Therefore, these data suggest that neuroleptic-induced alterations of SP-metabolizing neuropeptidases are mediated through additive interactions at multiple DA receptor subtypes. (Supported by NIMH grant MH42600 and an AFPE fellowship).

OPIOIDS: ANATOMY, PHYSIOLOGY AND BEHAVIOR III

726.1

LOCALLY ADMINISTERED MORPHINE REDUCES VENTRAL PALLIDAL OPIOID PEPTIDE RELEASE. M.F. Olive* and N.T. Maidment. Neuroscience Ph.D. Program & Dept. of Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024, USA.

Previous work from our laboratory has demonstrated a morphine-induced elevation in pallidal extracellular opioid peptide levels following peripheral administration (NeuroReport, in press). In an initial attempt to determine the site of action for this effect, morphine was locally administered into the ventral pallidum and changes in opioid peptide release in this region were studied in freely moving rats using in vivo microdialysis coupled to highly sensitive radioimmunoassay procedures. We have previously shown that the opioid peptides recovered from pallidal dialysates are primarily met- and leu-enkephalin (Neuroscience 33:549-557, 1989). Male Sprague-Dawley rats were implantated with guide cannulae and allowed at least four days of recovery prior to implantation with dialysis probes (2 mm membrane length) into the ventral pallidum. Following an overnight equilibration period, dialysis samples were collected at 30 min intervals. When morphine was incorporated into the perfusion medium for 30 min at 2 h intervals, opioid peptide release was slightly but not significantly decreased with respect to baseline during each 90 min post-injection period at 10 μM (16.7±18.5%, n=7) and 100 μM (30± 15.3%, n=6) doses. However, when 100 μM morphine was incorporated into the perfusion medium for 120 min at 4 h intervals, opioid peptide release was significantly reduced during each 2 h period post-injection, with the second administration producing a more profound (46.9±14.9%, n=6) decrease. These data demonstrate that opioid peptide release in the ventral pallidum is suppressed by local administration of morphine and suggest that this peptide release is perhaps under control of presynaptic μ opioid receptor autoregulatory mechanisms. Supported by DA05010, DA05634 & the W.M. Keck Fdn.

726.2

INTRASTRIATAL MORPHINE DECREASES THE RELEASE OF DOPAMINE IN THE STRIATUM: TOLERANCE PRODUCED BY REPEATED MORPHINE ADMINISTRATION. T.P. Piepponen, J. Mikkola, M. Ruotsalainen & L. Ahtee*. Dept. of Pharmacy, Div. of Pharmacology and Toxicology, P.O.Box 15, FIN-00014 University of Helsinki, Finland.

It is well-known that systemic administration of opioids increases the synthesis, metabolism and release of dopamine (DA) in the striatum thereby increasing DA transmission. It appears, however, that morphine can also have an inhibitory effect on nigrostriatal DA release as reflected in striatal 3-methoxytyramine in rats (Ahtee et al., J. Pharmacol. Exp. Ther. 1990, 255, 803-808). The effect of morphine challenge on DA transmission is augmented during withdrawal from chronic morphine treatment (Ahtee et al., J. Pharmacol. Exp. Ther. 1989, 249, 303-310; Attila and Ahtee, N-S Arch. Pharmacol. 1984, 327, 201-207; Honkanen et al., Neurosci. Lett. 1994, 180, 119-122). Therefore, it is possible that the sensitization of DA release during withdrawal results from tolerance to the inhibitory effect of morphine on DA release. To study this inhibitory effect rats were implanted with microdialysis probes into the striatum under pentobarbitone anesthesia, and allowed to recover for 40 h after surgery. Morphine (1, 10, and 100 μM) was dissolved in Ringer-solution and given directly into the striatum through the microdialysis probe (2 μL/min) for 4 h. Extracellular concentrations of DA and its metabolites DOPAC and HVA were determined by HPLC with electrochemical detection. Morphine significantly and dose-dependently reduced striatal DA release (by up to 60 %, $P<0.001$). The concentrations of DA metabolites were only slightly decreased (by 10-15 %). The effect of morphine (100 μM) was reversed by naltrexone (2.5 mg/kg s.c.). In rats withdrawn for 48 h from repeated morphine treatment (20 to 50 mg/kg s.c. for 7 days) morphine (100 μM) reduced the release of DA significantly less than in rats treated repeatedly with saline ($P<0.01$). Our results confirm the assumption that there is an inhibitory component to the effect of morphine on striatal DA release. Further, development of tolerance to the inhibitory effect of morphine on striatal DA release may at least partly account for the sensitization of nigrostriatal DA transmission to morphine challenge during withdrawal. (Supported by grants from the Finnish Cultural Foundation, the University of Helsinki and the Academy of Finland)

726.3

POTASSIUM AND 4-AMINOPYRIDINE EVOKED RELEASE OF STRIATAL DOPAMINE AND HIPPOCAMPAL NOREPINEPHRINE DURING MORPHINE WITHDRAWAL. Kenneth Grasing*, Stefan D. Schlussman, and John J.Woodward Program in Clinical Pharmacology, Dept. of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, New Jersey 08901

Microdialysis studies have shown that opiate withdrawal can diminish *in-vivo* levels of extracellular dopamine (DA). Using a striatal slice preparation, previous studies have demonstrated that the electrically evoked release of DA is decreased for periods of up to three weeks following opioid withdrawal. We attempted to extend these findings by examining the potassium (K^+) and 4-aminopyridine (AP) stimulated release of DA in the striatum and norepinephrine (NE) release in the hippocampus following withdrawal from morphine dependence.

Wistar rats received continuous infusions of morphine sulfate (MS) at 6.4 mg/kg-hr delivered by subcutaneous osmotic pumps (n= 6) or sham operations (n = 6) for 7 days. Following 2 to 15 days of withdrawal, animals were sacrificed by decapitation, the striatum and hippocampus were removed and cross cut to 350 μm slices with a McIlwain tissue chopper. Tissue slices were washed extensively and then loaded with [^{3}H] neurotransmitter. After determining levels of baseline release, slices were exposed to 25 mM K^+ or 300 μM AP (stimulation 1[S1]), with a second release stimulated after an additional baseline period (S2). Tissue from morphine (MS) treated and control (CTL) subjects demonstrated similar levels of neurotransmitter release. Mean and standard deviation for striatal tissue S1 was 9.5% ± 3.4 and 10.4 ± 2.1 for CTL and MS K^+ stimulated with 6.0% ± 2.1 and 7.8% ± 3.9 for CTL and MS AP stimulated. Values for hippocampus were 7.5% ± 2.3 and 7.0 % ± 0.9 for CTL and MS K^+ stimulated and 3.9% ± 1.0 and 4.7% ± 1.7 for CTL and MS AP stimulated. In conclusion, niether K^+ or AP evoked release of striatal DA or hippocampal NE were altered during MS withdrawal.

726.4

EFFECT OF NITRIC OXIDE SYNTHASE INHIBITION ON ACUTE MORPHINE AND CLONIDINE WITHDRAWAL RESPONSE IN THE RAT LOCUS COERULEUS. S. Hall, S. Duggan, B. Milne, K. Jhamandas*. Departments of Pharmacology & Toxicology, and Anesthesia, Queen's University, Kingston, Ontario, Canada. K7L 3N6.

Activation of NMDA and non-NMDA receptors contributes to the hyperactivity of noradrenergic neurons of the locus coeruleus (LC) associated with opioid and non-opioid drug withdrawal syndromes. Using an *in vivo* voltammetric approach, we have examined the role of nitric oxide (NO), which mediates NMDA receptor function, in this hyperactivity. In the anesthetized rat, an acute intraventricular injection of morphine (10 μg) or clonidine (10 μg) suppressed catechol oxidation current (CA.OC) recorded from the LC using differential normal pulse voltammetry. A subsequent injection of the opioid antagonist naloxone (2 mg/kg IV) given 45 minutes after morphine, or the α_2-receptor antagonist atipamezole given 45 minutes after clonidine, reversed drug-induced inhibition of LC response and produced an increase in CA.OC above baseline (LC withdrawal response). Pretreatment of animals with Nω-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase, blocked the LC withdrawal response. This treatment, however, did not influence the inhibitory effect of morphine or clonidine action on LC activity. The results suggest that NO plays a role in the genesis of acute morphine and clonidine withdrawal at the level of LC. [Supported by Medical Research Council of Canada]

726.5

EFFECTS OF THE NO SYNTHASE INHIBITOR, L-NAME ON MORPHINE TOLERANCE: AN EEG STUDY IN THE RAT X. Guo, L. R. King, A. Mattia and J. E. Moreton*. Pharmacology and Toxicology, Univ. Maryland Sch. Pharmacy, Baltimore, MD 21201.

A previous study has shown that N^G-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, attenuated the development of tolerance to the analgesic effects of morphine in mice (Majeed et al., 1993). In this study, electroencephalography (EEG) was used to investigate the effect of L-NAME on morphine tolerance in three groups of female Sprague-Dawley rats bearing chronic i.v. cannulas and cerebrocortical electrodes. Groups I, II and III received chronic saline, chronic morphine, or chronic morphine plus L-NAME (5 mg/kg/day), respectively. Cumulative dose-response curves for EEG total power and edge frequency were generated in each group by giving morphine (2.5, 5, 10, 20, 40 and 80 mg/kg, i.v.). Relative to Group I, Group II demonstrated morphine tolerance as shown by decreased EEG total power and increased edge frequency. Coadministration of L-NAME (Group III) did not prevent morphine tolerance except at the highest morphine dose. Similar to the report of Rauhala et al. (1994) for hyperthermic and hormonal responses in the rat, our data likewise suggest that tolerance to the EEG effects of morphine are not attenuated by L-NAME.

726.6

5-NITRO-6,7-DIMETHYL-2,3-QUINOXALINEDIONE (ACEA-1328), A NOVEL NMDA RECEPTOR/GLYCINE SITE ANTAGONIST BLOCKS CHRONIC TOLERANCE TO MORPHINE IN THE TAIL FLICK TEST. **K. Lutfy[1], P. Doan[1], S.X. Cai[2] and E. Weber[1]. [1]Dept. of Pharmacology, University of California, Irvine, Irvine, CA 92717; [2]Acea Pharmaceuticals, Inc., A subsidiary of CoCensys, Inc., 213 Technology Drive, Irvine, CA 92718.**

The results of recent studies suggest that activation of the N-methyl-D-aspartate (NMDA) receptor is critical in the genesis of tolerance to morphine. In the present study we have tested the hypothesis that blockade of NMDA receptors via antagonism of the glycine co-agonist site can block opioid tolerance. To study the acute effect of ACEA-1328 on morphine-induced antinociception, mice were injected with either Bis-Tris (0.2 M) or ACEA-1328 (1, 10, and 20 mg/kg, i.p.). Mice were then immediately injected with morphine (1-8 mg/kg, s.c.), and tested in the tail flick assay 30 min later. In tolerance studies, mice were daily injected with either Bis-Tris or ACEA-1328 (20 mg/kg, i.p.) immediately followed by an injection of either saline or morphine (10 mg/kg, s.c.) for 9 days. On day 10 (test day), a baseline (pre-drug) tail flick latency was measured for each mouse; mice were then injected with morphine (3-10 mg/kg, s.c.). Thirty min later, mice were tested for post-drug tail flick latencies. ACEA-1328 acutely potentiated but chronically had no significant effect on the antinociceptive effect of morphine. Chronic administration of morphine produced an approximately 2-fold decrease in the potency of morphine which indicates tolerance developed to the antinociceptive effect of the drug. Co-administration of ACEA-1328 with morphine blocked morphine tolerance. These data suggest that blockade of the NMDA receptor/glycine co-agonist site can prevent morphine tolerance. (Supported in part by NIDA Grant DA 06726)

726.7

THE EFFECT OF MK-801 ON THE EXPRESSION OF MORPHINE TOLERANCE IN THE NEONATAL RAT SPINAL CORD. J.A. Bell* and C.L. Beglan. Neuroimaging & Drug Action Sec, IRP, NIDA, Balto, MD 21224.

We have previously shown chronic co-treatment with MK-801 does not prevent the development of morphine tolerance in the isolated spinal cord of the neonatal rat. In the present study we tested the effect of acute MK-801 on the expression of tolerance in this preparation. Acute morphine reduces the area of the ventral root potential (VRP) without reducing the peak amplitude. MK-801 (300nM) alone has no effect on the peak amplitude or area of the VRP, but enhances the morphine induced depression in saline treated neonates. Thompson et al., (1992) have characterized various components of the electrically-evoked VRP relating to various types of afferent input. In the present study we evaluated the peak of the prolonged component of the VRP which occurs 2 seconds after a single shock stimulation of the dorsal root of sufficient intensity to activate C-fibre afferents. MK-801 (300nM) alone had no effect on the amplitude of the VRP 2 seconds after the stimulus. Morphine in concentrations of (10nM, 100nM, 1uM, 10uM) also had no effect on the peak of the prolonged component; however, morphine reduced the area of the VRP by reducing the prolonged late portion of the response. Acute MK-801 with morphine in the above concentrations, decreased the 2 second peak by 23, 37, 55, 69 percent respectively in saline treated neonates. In chronic morphine treated neonates acute MK-801 (300nM) with morphine in the above concentrations decreased the 2 second peak by 11, 34, 53, 64 percent respectively. These results show, in the presence of morphine tolerance, acute MK-801 still acts in synergy with morphine to reduce the peak of the prolonged VRP. Thus tolerance to this synergistic effect is not produced by chronic morphine administration.

726.8

INTRACEREBRAL INJECTION OF MORPHINE IN THE SUBSTANTIA NIGRA INDUCES C-FOS PROTEIN IN THE STRIATUM ASSOCIATED WITH A ROTATIONAL BEHAVIOUR. B. Bontempi, J. Liu, S. S. Massa* and F.R. Sharp. Dept. of Neurology, Univ. of California at San Francisco and SFVAMC, San Francisco, CA 94121.

We previously showed that systemic adminstration of morphine induced c-fos mRNA and fos protein in neurons of the medial and ventral striatum (ST) and in the nucleus accumbens. The induction was completely blocked by a pretreatment with the D1 antagonist SCH23390 and the NMDA receptor antagonist MK-801. (Liu et al.,1994, PNAS, 91, 8537-8541). However, these results do not demonstrate where morphine is actually acting nor what neuronal pathways and receptors are mediating the morphine induciton of fos. In an attempt to answer these questions, using standard stereotaxic surgical techniques, local intracerebral injections of morphine have been performed into brain regions hypothesized to be involved in mediating fos induction. The injection of 10 ug of morphine in the substantia nigra (SN) induces two different behavioral responses associtaed with two different patterns of Fos proteins in the ST. Some animals remain quiet after the injection and their fos pattern in the ST is similar to the one received systemic injection of morphine (refered as medio-dorso pattern). Others develop a rotation behaviour controlateral to the injection side, associated with striatal fos pattern much more latero-dorsal. The unilateral injection in the SN of the mu1 selective antagonist naloxonazine given 30 minutes prior to the systemic injection of morphine prevents c-fos induction in the ST ipsilateral to the naloxonazine injection, showing that morphine acts mainly through the mu1 receptors located in the SN to induce dopamine release and fos protein in the ST. Taken together, these results suggest tht fos induction reflects the activation of D1 receptors and could be used as a potential marker for dopamine release in particular areas of the ST that may be responsible for rotational behaviour.

726.9

EFFECT OF NALOXONE AND NEUROPEPTIDE FF (NPFF) ON THE EXPRESSION OF c-FOS IN THE BRAIN OF MORPHINE-DEPENDENT RATS. K.H. Jhamandas, K.H. Harris*, M. Sutak and J.H. Jhamandas. Department of Pharmacology & Toxicology, Queen's University, Kingston, Ontario & Department of Medicine (Neurology) & Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada.

NPFF has been designated as an endogenous morphine modulatory peptide with a potential to exert anti-opioid or opioid-like effects. To assess the potential of this peptide as an anti-opioid, we examined the effect of NPFF, 1DME (a synthetic NPFF analog resistant to degradation), and naloxone on behaviour and CNS expression of Fos in morphine-dependent and control rats. Animals received intracerebroventricular infusions of morphine (15μg/hr) or saline for 5 days, following which central injections of either naloxone, NPFF, or 1DME were made. In saline-infused animals naloxone produced few behavioural signs and minimal expression of brain Fos immunoreactivity. Injection of naloxone (2μg) into the lateral ventricle of morphine-dependent animals produced signs of autonomic and somatic hyperactivity. It also produced significant elevation of Fos positive neurons in the area postrema, nucleus tractus solitarius, locus coeruleus and paraventricular nucleus. Injection of NPFF (5μg) or 1DME (10μg) produced behavioural signs and induced Fos levels comparable to those seen after naloxone injection in saline-treated animals. These results suggest that centrally administered NPFF or its analog do not appear to mimick the actions of naloxone in morphine-dependent animals.

Supported by the Medical Research Council of Canada

726.10

CHRONIC EXPOSURE TO MORPHINE ATTENUATES THE EXPRESSION OF INTERLEUKIN-1β CONVERTING ENZYME IN THE RAT BRAIN. S.L. Chang*, G. Wu, J. Graf, [1]J.E. Zadina and N.A. Patel Department of Biology, Seton Hall University, South Orange, NJ 07079; [1]VA Medical Center and Tulane Medical School, New Orleans, LA

Alterations in the production, activity, or metabolism of interleukin-1β (IL-1β), an immunocytokine, by exogenous factors may have modulatory effects on the neuro-endocrine-immune system. We previously reported that chronic morphine treatment attenuates interleukin-1β activation of FOS expression in the rat hypothalamic paraventricular nucleus (Chang, et al 1994). Recently, we found that chronic exposure to morphine attenuates IL-1β immunoreactivity in the rat hippocampus (Patel, et al 1995). A cysteine protease, interleukin-1β converting enzyme (ICE), has been shown to cleave the inactive 31 Kd precursor to yield the biologically active 17 Kd form of IL-1β. In this study, we examined the expression of ICE by reverse transcriptase polymerase chain reaction (RT-PCR) in the rat hypothalamus and hippocampus following chronic treatment with morphine versus placebo. Adult male Harlan Sprague-Dawley rats (275-300 g) were implanted subcutaneously with 2 pellets of either morphine sulfate (75 mg/pellet) or placebo on Day 1 and 4 pellets on Day 2. On Day 5, the animals were decapitated and the hippocampus and hypothalamus were collected and frozen on dry ice until total RNA isolation. The RT-PCR procedures were performed on the total RNA samples. The expression of ICE in both hypothalamus and hippocampus of the rat given chronic morphine treatment was less than that of animals given placebo. These data suggest that a decrease in ICE may mediate, or contribute to the attenuation of IL-1β expression in the rat brain following chronic treatment with morphine.

726.11

CHRONIC MORPHINE (MOR) TREATMENT LEADS TO COMPARABLE REDUCTIONS IN RESPONSES OF GUINEA PIG nTS NEURONS TO MOR, MUSCIMOL (MUS) AND 2-CHLOROADENOSINE (2-CADO). C.J. Malanga, W.W. Fleming and D.A. Taylor*, Dept. of Pharmacology, WVU School of Medicine, Morgantown, WV 26506-9223.

Chronic implantation with MOR pellet leads to a 5.6-fold subsensitivity of guinea pig nTS neurons ***in vitro*** to MUS and a 3-fold supersensitivity to elevations in extracellular K^+. Since cumulative addition of either MOR or 2-CADO leads to acute desensitization in control preparations, the specificity of the chronic subsensitivity of opioid tolerant nTS neurons was examined using single concentrations of the agonists. Extracellular action potentials of individual nTS neurons were recorded and drug responses quantified as the percent reduction in frequency. Each neuron was exposed to MOR (1μM) and either MUS (0.3μM) or 2-CADO (1μM). Chronic treatment with MOR led to a magnitude of reduction in the inhibitory activity of each agent that was similar among brain slice preparations for each agonist employed. MUS produced 50% while MOR produced 75% inhibition of neuronal activity in placebo treated preparations. Chronic treatment with MOR reduced the inhibitory activity of MOR by 41% and that of MUS by 47%. 2-CADO reduced neuronal activity by 65% while MOR inhibited the activity of the same neurons by 60%. Chronic ***in vivo*** treatment with morphine reduced the inhibitory effects of these nTS neurons ***in vitro*** by 66% for MOR and 65% for 2-CADO. Thus, the development of tolerance to opioids in the guinea-pig brainstem is associated with a nonspecific change in responsiveness to a variety of agonists. The data are consistent with a cellular mechanism of adaptation involving general neuronal responsiveness, such as membrane properties, rather than specific receptors. Supported in part by NIH grant DA03773.

726.12

NORADRENERGIC BLOCKADE DISSOCIATES BIOCHEMICAL AND NEUROSECRETORY RESPONSES OF RAT OXYTOCIN NEURONES TO MORPHINE WITHDRAWAL EXCITATION.

C.H. Brown*, L.E. Johnstone, T. Onaka†, N.P. Murphy, G. Leng & J.A. Russell.

Department of Physiology, University of Edinburgh, EH8 9AG, U. K., †Department of Physiology, Jichi Medical School, Japan, 329-04.

Magnocellular oxytocin (OT) cells develop morphine dependence, seen as increases in firing rate, supraoptic nucleus (SON) Fos protein expression and OT secretion following naloxone (NLX)-precipitated withdrawal (Bicknell *et al*, J. Physiol., 396, 297-317, 1988). Brainstem noradrenergic (NA) cells, principally the A2 group in the nucleus tractus solitarius (NTS), project to SON OT cells (Cunningham & Sawchenko, TINS, 14, 406-411, 1991) and also develop morphine dependence (Stornetta *et al*, Brain Res., 624, 19-28, 1993). Acute intracerebroventricular (i.c.v.) infusion of the α_1-antagonist, benoxathian (BEN), delays the increase in firing rate of SON OT cells and the increase in plasma OT concentrations following withdrawal. Thus, withdrawal excitation of OT cells may passively follow excitation in the afferent A2 projection. Expression of the protein product (Fos) of the immediate early gene *c-fos* was used to determine the activation of SON OT cells during NLX-precipitated withdrawal in barbiturate-anaesthetised Sprague-Dawley rats challenged with a prolonged infusion of BEN. Projections to the SON were retrogradely-labelled with fluorescent microspheres and this was coupled with Fos immunocytochemistry to determine which inputs to the SON are activated during withdrawal excitation. Contrary to the electrophysiological and plasma OT results, the increased Fos expression in the SON was unaltered by i.c.v infusion of BEN indicating that, while electrical and secretory activity is attenuated by α_1-antagonism, biochemical excitation progresses within OT cells regardless. Of retrogradely-labelled cells, NTS cells are selectively activated during withdrawal. Thus, dependence is an intrinsic property of OT cells but synaptic activity in afferent NTS NA projections is required for expression of withdrawal excitation as neurosecretion. Supported by the B.B.S.R.C.

726.13

MORPHINE-INDUCED ANALGESIA IS DIFFERENTIALLY AFFECTED BY DURATION AND TYPE OF SUCROSE FEEDING. K. E. D'Anci, R. B. Kanarek*, R. Marks-Kaufman and H. Blohm. Dept. of Psychology., Tufts Univ., Medford, MA 02155.

Previous research shows that intake of palatable foods alters morphine-induced analgesia (MIA). Some of these studies found an enhanced MIA after sucrose feeding whereas others showed a decreased MIA. The purpose of the present studies was to examine whether acute versus chronic exposure to sucrose solutions differentially alters MIA, and whether availability of water in addition to sucrose is necessary to enhance MIA.

Experiment 1 used 20 adult male Long-Evans VAF rats. All rats had ad lib access to ground chow and water. Ten rats were also given a 32% sucrose solution in addition to water. The acute test phase took place after 5 hr of access to either sucrose or water alone. The chronic test phase took place after rats had been consuming sucrose for 3 wks. MIA was assessed with the tail-flick method. Injections of 2.5 mg/kg (ip) of morphine sulfate were given in a cumulative dose paradigm every 30 min. until a final dose of 15 mg/kg was achieved. In the acute feeding test, sucrose suppressed MIA, whereas chronic intake of sucrose significantly enhanced MIA.

The second study examined if a choice between water and sucrose was necessary to alter MIA. Three diet conditions were used with 10 rats in each: water alone, sucrose and water, and sucrose alone. All rats were also fed ground chow. There was an acute test after 5 hr of fluid availability, and a chronic test after 3 wks. In the acute phase, sucrose alone suppressed MIA relative to water and sucrose and water. During the chronic stage, however, exposure to sucrose regardless of a water choice enhanced MIA.

These results indicate that duration of exposure to palatable foods influences MIA. Acute exposure may release endogenous opioid peptides and induce tolerance to morphine, while chronic intake may alter opioid receptors.

726.14

CHRONIC MORPHINE AND ACUTE ESTROGEN: CONVERGENCE ON μ-OPIOID RECEPTOR COUPLING TO THE INWARDLY-RECTIFYING K^+CHANNEL IN HYPOTHALAMIC ARCUATE NEURONS (ARC) . G. Zhang*,E.J. Wagner, A.H. Lagrange, O.K. Ronnekleiv and M.J. Kelly.

Department of Physiology, Oregon Health Sciences U., Portland, OR 97201.

We have demonstrated that chronic morphine treatment (7 days) causes tolerance of ARC neurons to μ-opioid receptor activation (*Reg Peptides* 54:145, 1994), and that acute 17-β estradiol (E_2) rapidly uncouples the μ-opioid receptor from its K^+ channel in a subpopulation of ARC neurons (*J. Neurosci* 14:6196, 1994). The aim of this study was two-fold: (1) to investigate the effects of a 4 day chronic morphine regimen on the potency and efficacy of μ-agonist DAMGO-mediated hyperpolarization of ARC neurons; and (2) to measure the acute effects of E_2 on ARC neurons from morphine-treated animals. Intracellular recordings were made in hypothalamic slices from ovariectomized guinea pigs (GP) implanted s.c. with morphine- or placebo-pellets (4x75 mg for 2 days plus 6 more for a total of 4 days). The EC_{50} and V_{max} of DAMGO elicited hyperpolarization in control (n=5) was 45±4 nM and 14±3 mV, respectively. Based on the analysis of DAMGO potency data using an ANOVA with post-hoc tests for the morphine-treated GP, the ARC neurons were divided into three distinct groups ($p < 0.01$): non-tolerant with an EC_{50} of 52 ±7 nM (n=7); tolerant with an EC_{50} of 117 ±11 nM (n=4); and super-tolerant with an EC_{50} of 241±30 nM (n=4) and a decreased V_{max}. This data from the 4-day regimen further confirm previous findings of multiple expression of tolerance to μ-opioids in ARC neurons with a 7 day-morphine treatment. Moreover, in morphine-tolerant animals there was no significant change (paired t-test, n=9, $p>0.05$) in the EC_{50} of DAMGO after acute exposure (20 min) to E_2 (100 nM). The apparent occlusion of E_2's actions by chronic morphine may provide valuable information for determining the underlying mechanisms of these two modulators (Supported by PHS Grant DA05158).

726.15

HOMOLOGOUS VS. HETEROLOGOUS DESENSITIZATION OF RECEPTOR-MEDIATED POTASSIUM CURRENTS IN THE RAT LOCUS COERULEUS. S.L Ingram*, C.D. Fiorillo and J.T. Williams. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

μ-opioid and α_2-adrenergic receptors both activate the same potassium channels in rat locus coeruleus (LC). Acute or chronic treatment with opioid agonists induces tolerance that is specific to the μ-opioid response, with little change in the α_2 mediated response. We have performed intracellular recordings to make a quantitative comparison of desensitization to opioid and α_2 agonists in a brain slice preparation of rat LC. Drug-induced outward potassium currents were measured in neurons voltage-clamped at -60 mV. A supramaximal concentration of the opioid agonist met-enkephalin induced a profound homologous desensitization, but little heterologous desensitization to α_2 agonists or somatostatin. A supramaximal concentration of the α_2 selective agonist UK14304 showed relatively little desensitization. Current-voltage plots revealed that both the opioid and α_2 desensitization observed at -60 mV was due only to a reduction in potassium current. Opioid and α_2 desensitization could also be distinguished by muscarinic modulation. In the presence of muscarinic agonists, the rate and magnitude of opioid desensitization was increased, while α_2 desensitization was not altered. The acute desensitization examined here shares a number of characteristics with the desensitization observed in chronically morphine treated animals and may play a role in the initiation of chronic tolerance to opioids.

726.16

INTERACTION BETWEEN OPIOIDS AND EXCITATORY NEUROTRANSMITTERS IN THE MEDIAL PREFRONTAL CORTEX. J.L. Giacchino* and S.J. Henriksen. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Mu opioids can mediate reinforcing effects of heroin in the rat. This mediation may involve alteration of endogenous neurotransmitter systems. Medial prefrontal cortex (mPFC) circuitry is a significant component of reinforcement pathways, and the activity of its neuronal population has been previously shown to be modified by both systemic and electrophoretically administered opioids. In this study we sought to determine possible mechanisms for this action.

Extracellular recordings were made from neurons in the mPFC in halothane-anesthetized rats. Areas investigated included the dorsal anterior cingulate, prelimbic and infralimbic components of the mPFC. Systemic morphine (2.5 mg/kg, sc) and electrophoretically-applied DAMGO, a mu opioid agonist (1 mM), were evaluated in terms of their effect on mPFC neuronal firing in response to electrophoretically-applied acetylcholine (ACh, 1M) and glutamate (Glu, 0.2M). As expected, prior to the application of opioids, many mPFC cells were found to increase firing rate in response to both ACh and Glu. Systemic morphine attenuated or blocked the Glu-induced excitation in the majority of these cells, whereas the ACh excitatory effect remained unaltered. However, some neurons exhibited no changes in response to ACh or Glu following systemic morphine. As noted previously, electrophoretic application of DAMGO decreased the spontaneous firing of most mPFC neurons. When applied concurrently with either ACh or Glu, this mu agonist was found to have different effects as a function of the individual mPFC neuron studied. DAMGO attenuated the response to ACh and Glu in some cells, but in other neurons it clearly blocked the excitatory Glu response without altering the response to ACh. In a third group of neurons, DAMGO was found to have no effect on either the ACh- or Glu-induced responses.
(Supported by SDAC #DA00201 and NIDA #DA08301)

726.17

BACLOFEN-INDUCED INHIBITION OF HYPOTHALAMIC NEURONS: PHARMACOLOGY AND POTENTIAL FOR CROSS-TOLERANCE WITH A μ-OPIOID AGONIST FOLLOWING CHRONIC MORPHINE. E.J. Wagner*, G. Zhang, A.H. LaGrange, O.K. Ronnekleiv and M.J. Kelly. Dept. of Physiology, Oregon Health Sci. Univ., Portland, OR 97201.

The γ-aminobutyric acid $(GABA)_B$ receptor agonist baclofen has prominent inhibitory actions in neuroendocrine regions of the mammalian CNS. For example, baclofen inhibits arcuate nucleus neurons by membrane hyperpolarization attributed to an increase in an inwardly rectifying K^+ conductance. This response is identical to that following activation of μ-opioid receptors, which couple to the same effector in this brain region. The purpose of the present study was to characterize the pharmacology of the baclofen response, and to determine if cross-tolerance develops with μ-opioid agonists following chronic morphine treatment (subcutaneous implantation of 4 pellets each containing ~5mg of morphine on day 0, followed 2 days later with the implantation of 6 additional pellets) for 4-7 days. To this end, intracellular recordings of arcuate nucleus neurons were made in coronal hypothalamic slices (450μm) prepared from ovariectomized female guinea pigs. At a concentration of 30μM, the $GABA_B$ receptor blockers CGP 35348 and 2-hydroxysaclofen shifted the baclofen dose-response curve to the right with an estimated K_i of 3.2±1.4μM (mean±SEM; n=3) and 15.8±4.9μM (n=2), respectively. Compared to placebo-treated controls, chronic morphine treatment reduced the potency of the μ-opioid receptor agonist DAMGO but not of baclofen. Taken together, these results show that: 1) the inhibitory effect of baclofen on arcuate nucleus neurons is consistent with its actions as a $GABA_B$ receptor agonist, and 2) chronic morphine exposure does not produce cross-tolerance between $GABA_B$ and μ-opioid receptor agonists. This latter finding suggests that convergence of the two receptors systems to the same effector may not be a sufficient criterion for cross-tolerance between receptor systems. (This work was supported by Grants DA05158 and DA07262).

726.18

ACTION OF MU-SELECTIVE OPIOIDS ON COMMISSURAL PATHWAY MODULATION OF DENTATE GYRUS CIRCUITRY. J.H. Mayer*, S.J. Henriksen. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037

In order to better understand the effects of mu-selective opioids on the physiology of the dentate gyrus of the hippocampus, we stimulated (conditioning stimulation) the contralateral hilus (CH) while concurrently recording single neurons and population spikes (PSs), evoked by stimulation of the ipsilateral perforant path (PP), extracellularly in halothane anesthetized rats. Single cells were characterized electrophysiologically as dentate granule cells (DGCs), interneurons (INTs), or hilar mossy cells (HMCs). Subsequently, [D-Ala^2, NMe-Phe^4, Gly-ol]-Enkephalin (DAMGO), a mu-selective opioid, was administered electrophoretically. We found that stimulation of the commissural pathway prior to stimulation of the PP reduced the PS amplitude. This effect was dependent on the stimulus intensity and the interval between the two stimuli. CH stimulation by itself did not produce a PS and individual DGCs could not be driven. However, CH stimulation orthodromically activated INTs, both in the granule cell layer and within the hilus. HMCs were typically either not activated by CH stimulation, or were driven antidromically. Administration of DAMGO tended to reverse the inhibition produced by CH stimulation on perforant path PSs; however, this effect was less impressive when the stimulus intensity at the PP was adjusted to control for the primary PS amplitude. DAMGO did not affect the latency of INTs to activation by CH stimulation at stimulus intensities that were relevant for inhibition of the PS. We conclude that stimulation of the commissural pathway reduces PP-evoked PSs through the excitation of INTs, both within the granule cell layer and the hilus. Furthermore, the influence of mu-selective opioids on this pathway is not as pronounced as the mu-related effects on the PP or on the spontaneous activity of cells within the dentate gyrus. (Supported by DA 00143 to JHM)

726.19

cAMP CASCADE: ROLE IN μ OPIOID-MEDIATED INHIBITION OF NMDA CURRENTS IN DENTATE GRANULE CELLS. C.W. Xie[1]* and D.V. Lewis[2] [1] Dept. Psychiatry, UCLA, Los Angeles, CA 90024. [2] Dept. Pediatrics, Duke Univ. Med. Ctr, Durham, NC 27710

We have previously reported that activation of μ opioid receptors inhibited NMDA-mediated synaptic currents in dentate granule cells. The present study further characterized this effect and examined possible involvement of the cAMP cascade. Synaptic currents were evoked by stimulating the dentate outer molecular layer, and recorded from granule cells using whole-cell voltage-clamp techniques in the hippocampal slice preparation. NMDA receptor-mediated excitatory postsynaptic currents (NMDA EPSCs) were isolated in the presence of the AMPA antagonist DNQX and the $GABA_A$ antagonist bicuculline methiodide. A dose-dependent reduction in the amplitude of NMDA EPSCs was observed following bath application of the μ agonist PL017 (0.3 -10 μM). This reduction seemed not dependent upon the presence of extracellular Mg^{2+}, and could be effectively prevented by pertussis toxin included in the recording pipette. In contrast, the adenylate cyclase activator forskolin (10-100 μM) was found to increase the amplitude of NMDA EPSCs in a dose-dependent manner. Forskolin (100 μM) also significantly attenuated PL017-induced reduction of NMDA currents. These results suggested that the cAMP cascade may be involved in μ opioid-induced inhibition of NMDA currents.

726.20

DEMONSTRATION OF μ-OPIOID RECEPTOR SUPPRESSION OF Ca^{2+} CURRENTS IN CENTRAL NEURONS. B.L. Soldo* and H.C. Moises. Department of Physiology, University of Michigan, Ann Arbor, MI 48109.

Opiates and opioid peptides have been shown to suppress voltage-sensitive Ca^{2+} conductances in peripheral neurons and a variety of neuron-like cell lines; however, this effect has only recently been demonstrated in central neurons. In this study, whole-cell Ca^{2+} channel currents were recorded from acutely isolated neurons of rat ventral forebrain, a region rich in opiate receptors, to examine the possible coupling between opioid receptors and neuronal Ca^{2+} channels. High voltage-activated (HVA) currents were elicited by 100 ms depolarizing steps (-10 mV to +10 mV) from a holding potential of -80 mV, using 4mM Ba^{2+} as charge carrier. Bath application of the μ-opioid selective agonist H-Tyr-D-Ala-Gly-Phe(N-Me)-Gly-ol (DAGO, 3μM) reversibly suppressed the peak amplitude of HVA currents (with 40% maximal effect) and slowed their rate of activation. The L- and N-type Ca^{2+} channel blockers, nifedipine and ω-conotoxin GVIA, respectively, blocked pharmacologically distinct components of the HVA current in these neurons. The DAGO-sensitive component was reduced after blockade of N-type currents by GVIA. In addition, cells that showed Ca^{2+} inhibitory responses to DAGO exhibited morphological features of principal neurons and stained positive for acetylcholinesterase. These data suggest that postsynaptic μ-opioid receptors are negatively coupled to N-type Ca^{2+} channels in rat forebrain cholinergic neurons. (Supported by NIH grant DA-03365).

SEROTONIN RECEPTORS: 5-HT_1

727.1

BLOCKADE OF 5-HT_{1A} RECEPTORS EXPRESSION USING LOCAL INJECTIONS OF ANTI-SENS OLIGONUCLEOTIDES. M.-C. Miquel[1], Y. Sari[1], C. Sibella[1], H.K. Kia[1], G. Daval[1], B. Emerit*[2], M. Hamon[2] and D. Vergé[1]. [1]CNRS URA 1488, Univ. Paris VI, 75005 Paris. [2]INSERM U288, CHU Pitié-Salpêtrière, 75013 Paris, France.

5-HT_{1A} receptors (5-HT_{1A}-R) are involved in a lot of various physiological functions. 5-HT_{1A}-R are principally localized postsynaptically in the limbic structures and in the raphe nuclei as somato-dendritic autoreceptors. With the aim of further analyzing the role of 5-HT_{1A}-R in each of their precise localizations, we have locally blocked their expression in the adult rat, using anti-sens oligonucleotides (ONs). Different ONs, complementary to the N-terminal portion of the mRNA coding for 5-HT_{1A}-R, were designed as either unmodified, phosphorothioate or 3'end-alkylamine-attached ONs.

In order to validate this approach, stereotaxic injections were performed in the dorsal hippocampus using mixtures of ONs (anti-sens, sens or scrambled) and lipofectin. The mixtures were delivered either in single or repeated injections, or in a continuous mode using Alzet osmotic minipumps. The resulting effect on 5-HT_{1A}-R density was analyzed by receptor quantitative autoradiography. Specificity and histological controls were performed.

Experiments using single injections (2.5 μg/2 μL) demonstrated that the 3'end-alkylamine-attached ONs were the most efficient, giving up to 60% decrease of 5-HT_{1A}-R density. The unmodified ONs were almost inefficient and phosphorothiate ONs exhibited cytotoxicity. Maximum effect was observed 4 days after injection.

Another approach using plasmids containing anti-sens RNAs is currently under investigation in order to obtain a continuous blockade for a longer period of time. Both approaches will be used to block the expression of 5-HT_{1A} autoreceptors in the raphe nuclei to assess their specific role in anxiety.

727.2

IN VITRO AND *IN VIVO* ALKYLATION OF CENTRAL 5-HT1A RECEPTORS. F. Radja*, E.K. Nénonéne, M. Carli and T.A. Reader. *CRSN, Département de physiologie, Faculté de Médecine, Université de Montréal, (Qc) Canada.*

Saturation binding of the serotonin agonist [^{3}H]8-OH-DPAT in cerebral cortex and hippocampus is best fitted to a two-site model, indicating an *heterogeneity of receptors* or several binding *affinitity states*. For cerebral cortex the affinities are of about 0.7 n*M* and 36 n*M*, and for hippocampus of 0.8 n*M* and 22 n*M*. Also, binding of 1 n*M* [^{3}H]8-OH-DPAT is inhibited by several 5-HT agonists and antagonists and, except for ritanserin, all competition curves are best described by a two-site model. The *in vitro* treatment of the membranes with *N*-ethylmaleimide (NEM) to alkylate sulfhydryl groups causes dose-dependent decreases of [^{3}H]8-OH-DPAT binding; all the inhibition curves are biphasic and the effects irreversible. After *in vivo* alkylations, carried out by treating rats with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydro-quinoline (EEDQ; 10 mg/Kg i.p.), the saturation curves from both control and EEDQ-treated rats are best fitted to a two-site model. However, for EEDQ-treated animals there is a drastic decrease in 5-HT1A receptors; this loss is greater in the hippocampus than in the cerebral cortex. In contrast, the low-affinity binding sites remained unchanged, indicating that this site is not a *G* protein coupled receptor. Since the decrease in 5-HT1A receptors is not associated with changes in low-affinity binding, the results suggest independent regulations of the two [^{3}H]8-OH-DPAT binding sites. Altogether, the present data further supports the concept that [^{3}H]8-OH-DPAT, besides labelling 5-HT1A receptors, also binds to other sites in rat cerebral cortex and hippocampus.

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727.3

NCS-MPP (4-(2'-METHOXY-PHENYL)-1-[2'-(N-2"-PYRIDINYL)-P-ISOTHIOCYANOBENZAMIDO]-ETHYL-PIPERAZINE): A HIGH AFFINITY AND IRREVERSIBLE LIGAND FOR 5-HT$_{1A}$ RECEPTORS. H. F. Kung*, M.-P. Kung, M. Mu and Z.-P. Zhuang. Departments of Radiology and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

The successful development of a new pure 5-HT$_{1A}$ receptor antagonist, *p*-MPPI, provides a fresh impetus in developing affinity labels to further characterize the physiological roles and to elucidate the molecular structure of 5-HT$_{1A}$ receptors. An irreversible binding ligand, NCS-MPP (4-(2'-methoxy-phenyl)-1-[2'-(n-2"-pyridinyl)-p-isothiocyanobenzamido]-ethyl-piperazine), was prepared, and its binding characteristics were evaluated in *in vitro* binding with rat hippocampal membranes. The inhibition of NCS-MPP for [I-125]*p*-MPPI binding to 5-HT$_{1A}$ receptors resulted in a K_i = 1.8 ± 0.2 nM. NovaScreen of NCS-MPP exhibited low binding affinities to *alpha* -1, *alpha*-2, *beta* and 5-HT$_2$ receptors (K_i = 350, 420, >1000 and 103 nM, respectively). These data strongly suggest that the ligand bound to 5-HT$_{1A}$ receptors with high affinity and high selectivity. Irreversible inhibition of [I-125]*p*-MPPI binding by NCS-MPP, following a 5 min incubation at room temperature, was concentration dependent; the inhibition increased to 50% at the concentration less than 10 nM and became more pronounced (~90%) at 400 nM. Under similar assay conditions, NCS-MPP was significantly less efficient in irreversibly inhibiting agonist [I-125]8-OH-PIPAT (<10 nM). After pretreatment of membranes with a low concentration of NCS-MPP (2 nM), there was an apparent loss of [I-125]*p*-MPPI binding sites, as expected, but no change in binding affinity (K_d). However, the significant increase in K_d at a higher concentration of NCS-MPP (50 nM) indicated that there may be a secondary alkylation site not directly involved in *p*-MPPI binding; nevertheless, it would increase K_d. The irreversible ligand, NCS-MPP, may provide a useful tool for studies of 5-HT$_{1A}$ receptors in central nervous system. (supported by NS-24538 and MH-48125)

727.4

AGE-RELATED ALTERATIONS IN SEROTONERGIC RECEPTOR FUNCTION IN FOREBRAIN REGIONS FOLLOWING EEDQ TREATMENT. B.J. Keck* and J.M. Lakoski. Departments of Pharmacology & Anesthesia, The Pennsylvania State University College of Medicine, Hershey, PA 17033.

EEDQ (1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) is a neurotoxin which binds irreversibly to serotonin (5-HT) receptors and renders them permanently inactive; it is useful for investigating spare receptor reserves. Spare receptor capacities of 5-HT receptor subtypes have not yet been investigated with aging. Female Fischer 344 rats (3 and 22 mo) treated with EEDQ (6 mg/kg, s.c.) or vehicle (ETOH/propylene glycol/H_2O, 1:1:2, s.c.) 24 hr prior to sacrifice were used to examine changes in 5-HT$_{1A}$ function with age. Scatchard analyses using the 5-HT$_{1A}$ ligand [^{3}H]8-OH-DPAT were conducted in forebrain regions which contain high densities of this receptor subtype.

A significant decline in [^{3}H]8-OH-DPAT binding in hippocampal tissue was demonstrated in EEDQ treated rats of both age groups compared to controls (3 mo, $p=0.001$; 22 mo, $p=0.002$). Both ages were equally sensitive to the toxin-induced decrease (65%) in B_{MAX} for 5-HT$_{1A}$ hippocampal sites. In frontal cortex the 3 mo, but not the 22 mo, group demonstrated a significant reduction (70%, $p=0.02$) in 5-HT$_{1A}$ sites. K_D values were increased following EEDQ treatment in the hippocampus and frontal cortex in both ages. While a fourfold increase in the K_D was observed in the 3 mo group, an eightfold increase was demonstrated in the 22 mo group.

Overall, significant changes in B_{MAX} and K_D values at the 5-HT$_{1A}$ receptor were observed following EEDQ treatment. Additionally, site-dependent differences were revealed in age-related responses to EEDQ. Using quantitative autoradiographic techniques, studies of the aging 5-HT$_{1A}$ receptor will be expanded to include analyses of brainstem raphe nuclei known to contain spare 5-HT$_{1A}$ receptors.

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727.5

IRREVERSIBLE BLOCKADE OF 5-HT$_{1A}$ RECEPTORS *IN VITRO* BY A SELECTIVE ALKYLATING AGENT. S. Bhamre[1*], H.F. Kung[2] and A. Frazer[1]. [1]Dept. of Pharmacol, UTHSCA, San Antonio, Texas 78284 and [2]Dept. of Radiol & Pharmacol, Univ of PA, Philadelphia, PA 19104.

Using an irreversible alkylating agent, EEDQ, it has been inferred that there is a greater receptor reserve at somatodendritic 5-HT$_{1A}$ receptors than at postsynaptic ones (Mol Pharmacol 41:1066, 1992). However, results with EEDQ need to be interpreted cautiously as it is nonselective. We have evaluated the effects *in vitro* of a novel selective alkylating agent 4-(2'-methoxyphenyl)-1-[2'-(N-2"-pyridinyl)-p-isothiocyanobenzamido]ethylpiperazine (p-MPPSCN). p-MPPSCN has at least 10 fold greater affinity for 5-HT$_{1A}$ receptors than for other biogenic amine receptors. The binding of [^{3}H]8-hydroxy-2-(di-n-propylamino)tetralin ([^{3}H]8-OH-DPAT) to 5-HT$_{1A}$ receptor sites in rat hippocampal homogenates, using membranes that had been pre-incubated with either vehicle or p-MPPSCN, was studied. The membranes were washed 4 times before being added to the assay tubes. Concentrations of p-MPPSCN below 5nM did not inhibit the binding of [^{3}H]8-OH-DPAT, whereas concentrations of ≥ 10nM did. Analysis of saturation experiments carried out with [^{3}H]8-OH-DPAT revealed that p-MPPSCN (10 nM) caused no change in Kd (0.93±0.2, CTRL; 1.1±0.2 nM, p-MPPSCN), but decreased Bmax (237±20, CTRL; 151±5 fmol/mg protein, p-MPPSCN; $p<0.025$). Upon pre-incubation of membranes with WAY100135 (200 nM), a competitive antagonist of 5HT$_{1A}$ receptors, followed by washing, there was no inhibition of binding of [^{3}H]8-OH-DPAT. When membranes were pre-incubated with both p-MPPSCN (10nM) and WAY100135 (200nM), there was less inhibition of binding of [^{3}H]8-OH-DPAT (11± 6%) compared with that in membranes incubated with p-MPPSCN alone (36±9%; $p<0.05$). p-MPPSCN appears to irreversibly block 5-HT$_{1A}$ receptors *in vitro*. Its effects *in vivo* are currently under evaluation. (Supported by research funds from the VA and USPHS MH48125).

727.6

MOLECULAR CHARACTERIZATION OF ANTIPEPTIDE ANTIBODIES AGAINST RAT BRAIN 5HT$_{1A}$ RECEPTOR [1]T.E. Anthony, [1]N.M. Kheck, [2]P.R. Albert, [3]P.M. Whitaker-Azmitia, and [1]E.C. Azmitia*, and [1]Dept. of Biology, New York University, NY, NY 10003; [2]Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Canada H3G 1Y6; [3]Dept. of Psychiatry, SUNY, Stony Brook, NY 11794.

The cloning and sequencing of the rat 5HT$_{1A}$ receptor gene has allowed for the production of antipeptide antibodies against its receptor protein product. We are currently characterizing the localization of the 5HT$_{1A}$ receptor protein in the rat and primate brain, using antipeptide antibodies targeted against two distinct regions of the receptor which share low homology with other known proteins. The S1A170 is a polyclonal antibody raised in rabbit against amino sequence 170 - 186, a region spanning from the fourth transmembrane domain to the second extracellular loop, which is structurally linked to the agonist binding site. The S1A258 recognizes amino acids 258-274,a region within the third cytoplasmic loop that comprises the putative G protein binding site. Characterization of these antibodies employing SDS-PAGE and immunoblotting of adult hippocampal membrane preparations has shown the S1A170 antibody labels a major band at approximately 64 kDa. Immunoblots of LZD-7 fibroblasts transfected with the cDNA for the rat 5HT$_{1A}$ receptor have also shown this protein band, which was absent in Ltk- nontransfected cells. In the rat, developmental and brain regional differences have been noted through the differential intensites of the protein banding pattern obtained from immunoblots. In addition to molecular studies, immunocytochemical studies using these antibodies have demonstrated a pattern of 5HT$_{1A}$ receptor distribution in rat, cat and monkey brain that correlates well with previously reported radioligand binding sites. For example, in the brainstem raphe nuclei of these three species, 5HT$_{1A}$ receptor-IR is localized to the soma and dendrites of serotonergic neurons, thus labeling the known population of presynaptic autoreceptors. Our findings indicate that these site-directed antipeptide antibodies provide another specific means for the localization and quantification of 5HT$_{1A}$ receptor protein in the mammalian CNS. (NIA#:PO1-AG10208)

727.7

QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF 5-HT$_{1A}$ RECEPTORS USING AGONIST AND ANTAGONIST RADIOLIGANDS. P. A. Scott*& A. Frazer. Dept. of Pharmacology, UTHSCSA, and Audie L. Murphy Memorial. Veterans. Hospital, San Antonio, TX, 78284.

Only recently have radiolabeled antagonists for the 5-HT$_{1A}$ receptor become available. We have compared the binding characteristics of one such radioligand, ^{3}H-4-(2'methoxy-)phenyl-1-[2'-(n-2"-pyridinyl-)p-flourobenzamido-]ethylpiperazine (^{3}H-p-MPPF), with that of two agonist radioligands, ^{3}H-8-hydroxy-2-(di-n-propylamino)tetralin (^{3}H-8-OH-DPAT) and ^{3}H-(+)4-[n-5(methoxychroman-3yl)n-propylamino]butyl-8-azaspiro[4,5]decane-7,9 dione (^{3}H-(+) S-20499), using slide-mounted sections of rat brain. The radioactivity in each section was measured by either liquid scintillation spectrophotometry or by quantitative autoradiography. Optimal times of incubation were 60 min for ^{3}H-8-OH-DPAT and 90 min for ^{3}H-(+) S-20499 and ^{3}H-p-MPPF. To reduce non-specific binding with ^{3}H-(+) S-20499, it was necessary to wash the sections for 60 min at 4°C after incubation , rather than the 10 min that was sufficient for the other two radioligands. The K_D values for the three radioligands were about 0.6, 1.0, and 1.6 nM for ^{3}H-8-OH-DPAT, ^{3}H-(+) S-20499 and ^{3}H-p-MPPF respectively. Addition of Gpp(NH)p (10 μM) to the incubation medium reduced the binding of the two agonist radioligands (about 70% for ^{3}H-8-OH-DPAT and 50% for ^{3}H-(+) S-20499), but did not alter the binding of ^{3}H-p-MPPF. At the K_D concentration of ^{3}H-p-MPPF, nonspecific binding was 5% of total binding, which compared favorably with the 2% nonspecific binding of ^{3}H-8-OH-DPAT. By contrast, nonspecific binding of ^{3}H-(+) S-20499 was 15% of total binding. Autoradiographic analysis of the regional distribution in brain of the binding of the three radioligands showed a high correlation. The rank order of binding was entorhinal cortex ≥ dorsal raphe nucleus > dentate gyrus > CA2/3 > CA1. Only in the dorsal raphe nucleus did the binding of ^{3}H-p-MPPF appear to be greater than that of the two agonist radioligands. Supported by funds from the VA, USPHS Grant MH48125, and IRI Servier.

727.8

DISTRIBUTION OF GABA AND 5-HT1A IMMUNOPOSITIVE NEURONS IN THE RAT HIPPOCAMPUS. T. D. Patel* and F. C. Zhou. Medical Neurobiology Program and Dept. of Anatomy, Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

GABA and serotonin (5-HT) through the 5-HT1A receptor are major inhibitors of excitatory neurotransmission in the hippocampus. GABA interneurons send inhibitory input to the granule cells of the dentate gyrus (DG) and the pyramidal cells of Ammon's horn (CA). 5-HT also inhibits axonal firing of these cells through activation of the 5-HT1A receptor. Little is known regarding the interactions between these two systems regulating hippocamapal circuitry. In this study we used double immunocytochemistry to correlate the distribution of 5-HT1A receptors with GABA neurons and their terminal fibers in the rat hippocampus. GABA neurons were identifed using an antibody against glutamate decarboxylase (GAD), and 5-HT1A using an anti-peptide (a.a. 170-186 of rat 5-HT1A) antibody (Azmitia et al., 1992).

Consistent with our previous report (Patel et al., 1995), 5-HT1A-immunopositive (im) staining was most prominent in the DG granule cell layer and the CA pyramidal cell layer where it was detected on the initial segment of the axon. In contrast, fine GAD-im punctate terminals were distributed as dense clusters outlining the soma of granule and pyramidal cells. GAD-im cell bodies and processes with cytoplasmic staining were detected in the molecular and polymorphic layers of DG and stratum oriens, radiatum, and lacunosum moleculare of CA. Relatively sparse 5-HT1A-im cell staining was detected in these layers. Most cells were singly labeled for GAD or 5-HT1A. In the polymorphic layer 5-HT1A-im staining was found on large Mossy cells which send excitatory projections to the granule cells. Also in the DG, few scattered cells did exhibit both GAD and 5-HT1A immunoreactivity.

These findings suggest that in the hippocampus (1) 5-HT1A and GAD are mostly differentially expressed with little colocalization, (2) 5-HT and GABA may exert their inhibitory influence on the excitatory granule and pyramidal cells at spatially distinct subcellular sites, (3) 5-HT can indirectly influence granule cells through 5-HT1A receptors on Mossy cells, and (4) 5-HT may regulate some GABA neurons.

727.9

IMMUNO-ELECTRON MICROSCOPIC VISUALIZATION OF 5-HT$_{1A}$ RECEPTORS IN THE RAT BRAIN. D. Vergé[1], H.K. Kia[1], M.J. Brisorgueil[1], M. Hamon[2] and A. Calas*[1]. [1] CNRS URA 1488, Univ. Paris VI, 75005 Paris. [2] INSERM U288, CHU Pitié-Salpêtrière, 75013 Paris, France.

5-HT$_{1A}$ receptors (5-HT$_{1A}$-R) have been visualized in the rat brain with specific antibodies in various areas, including raphe nuclei and limbic regions. We used anti-peptide antibodies to investigate the localization of 5-HT$_{1A}$-R at the subcellular level in several of these areas.

Vibratome sections from rat brains were incubated with rabbit anti-peptide polyclonal antibodies against 5-HT$_{1A}$-R and processed for the ABC staining method. The peroxydase activity was revealed using DAB as a chromogen. Sections were then processed for electron microscopy.

The 5-HT$_{1A}$-R immunoreactivity (5-HT$_{1A}$-R-ir) was found exclusively in the somato-dendritic compartment of neurons and was never observed within glial processes. 5-HT$_{1A}$-R-ir could never be found in axons or nerve terminals. In the dorsal hippocampus, the immunolabeling was associated exclusively with dendrites. In the dorsal raphe nucleus as well as in the septal complex, 5-HT$_{1A}$-R-ir was found in dendritic processes and in somas. Some immunoreactivity was observed within the cytoplasm of cell bodies, but not in cell nuclei. In somas as well as in dendrites, 5-HT$_{1A}$-R-ir was unevenly distributed at the level of the plasmic membrane. It was frequently associated with synapses, but was also found extra-synaptically. This result indicates that the action of 5-HT via 5-HT$_{1A}$-R could occur through 'wire' as well as 'volumic' transmission. Non-labeled synapses were also observed. In the septal complex, choline acetyltransferase and 5-HT$_{1A}$-R could be found colocalized in the same neuron, in agreement with previous results indicating that serotonin can exert through 5-HT$_{1A}$-R a functional control of part of the septal cholinergic neurons which project to the hippocampus.

727.10

DIFFERENTIAL EXPRESSION OF SEROTONIN RECEPTORS 5-HT1Dα AND 5-HT2A mRNA IN HUMAN BRAIN VESSELS, VASCULAR CELLS AND ASTROCYTES IN CULTURE. Z. Cohen*, I. Bouchelet, W.V. Yong, J.-G. Villemure, D. Stanimirovic[1], and E. Hamel. Montreal Neurol Inst, McGill Univ, Montréal, QC and [1]National Research Council of Canada, Ottawa, ON, Canada.

Intraparenchymal blood vessels in the cerebral cortex are innervated by serotonin nerve terminals (Cohen *et al.*, 1995, *Neurosci.*, in press) and stimulation of their cells of origin affects cerebral blood flow and possibly blood brain barrier functions. However, the type and cellular localization of the 5-HT receptor(s) involved in these effects remain unknown. We studied the expression of 5-HT1Dα and 5-HT2A receptors mRNA in 1) freshly isolated human brain microvessels (MVs) and capillaries (CAPs) from post-mortem cortices, 2) subcloned endothelial (HBEC) and primary cultures of smooth muscle (HBSM) cells, respectively dissociated from CAPs (< 112 μm) and MVs (> 112 μm or > 350 μm) isolated from temporal cortex biopsies, and 3) astrocytes cultured from human fetal brain (HFBA). Tissue RNA was extracted, reverse transcribed, and submitted to polymerase chain reaction (PCR) using receptor-specific oligonucleotide primers. The effects of 5-HT on IP_3 formation and intracellular Ca^{+2} in HBEC were also evaluated. MVs, CAPs, and HBEC were enriched in γ-glutamyl transpeptidase and alkaline phosphatase; HBEC and HFBA were selectively immunoreactive to Factor VIII and GFAP, respectively. Gel electrophoresis of the PCR products showed expression of mRNA for the 5-HT1Dα in MVs (n=3), CAPs (n=4), HBEC (n=3 of 5), HBSM (n=2 of 3) but in only one of three HFBA preparations. Conversely, PCR using 5-HT2A primers yielded products of the expected size in MVs (n=4), CAPs (n=4), and HFBA (n=2 of 3) but not in any HBEC or HBSM cell preparations. 5-HT (1-100 μM) failed to alter IP_3 production or intracellular Ca^{+2} levels in HBEC. Altogether, the data suggest expression of 5-HT1Dα mRNA in brain vascular elements whereas 5-HT2A receptors appear predominantly expressed in astrocytes, including those associated with isolated MVs and CAPs. Further investigation should clarify the role of these 5-HT receptors in microvascular physiology. Supported by MRC and NRC of Canada and a McGill Major Studentship (Z.C.).

727.11

EXPRESSION OF SUMATRIPTAN-SENSITIVE SEROTONIN RECEPTORS mRNA IN HUMAN NEURONAL AND VASCULAR TISSUES. I. Bouchelet*, Z. Cohen, B. Case[1], P. Séguéla and E. Hamel. Montréal Neurological Institute and [1]Royal Victoria Hospital, Montréal, Québec, Canada H3A 2B4.

Sumatriptan is highly effective in the treatment of acute migraine attack. This effect has been related to serotonin 5-HT1Dα receptors on dural trigeminovascular afferents (Rebeck *et al.*, PNAS.,1994, 91: 3666.) and/or 5-HT1Dß receptors in cerebral blood vessels (Hamel *et al.*, Mol. Pharm., 1993, 44: 242.). A better knowledge of sumatriptan-sensitive 5-HT receptors in neuronal vs vascular tissues appears important for the understanding of migraine treatment. We studied the expression of mRNA coding for 5-HT1Dα, 5-HT1Dβ and 5-HT1F receptors in post-mortem human trigeminal ganglia (TG) and pial vessels (PV) using RT-PCR and receptor-specific oligonucleotide primers. In TG preparations, PCR products of the expected sizes for 5-HT1Dα and 5-HT1Dß receptor subtypes were both identified. In preparations from PV and from one coronary artery, PCR products corresponding to the 5-HT1Dß subtype were present while only an occasional and faint signal was detected for the 5-HT1Dα subtype. Preliminary experiments on TG and PV preparations suggested the presence of 5-HT1F mRNA in both tissues. Altogether, these results show that multiple sumatriptan-sensitive 5-HT receptors mRNA are co-expressed in neuronal and vascular tissues. Further, they may provide a basis for the sumatriptan-associated peripheral side-effects. Complementary studies by *in situ* hybridization are required to define the respective localizations and density of neuronal populations expressing these 5-HT receptors in putative target tissues for migraine therapy. Supported by the Québec Heart and Stroke Foundation.

727.12

5-HT$_{1A}$ AGONIST AND ANTAGONIST BINDING IN RAT BRAIN AND SPINAL CORD FOLLOWING 5,7-DHT TREATMENT. R. K. Raghupathi*, A. Singh, I. Lucki, M. -P. Kung, H. Kung and P. McGonigle. Departments of Pharmacology, Psychiatry and Radiology, University of Pennsylvania, Philadelphia, PA 19104-6084.

Changes in rat brain and spinal cord 5-HT$_{1A}$ receptors following 5,7-DHT treatment were examined using an agonist ligand [^{125}I]8-OH-PIPAT (PIPAT) as well as a newly developed antagonist ligand [^{125}I]-p-MPPI (MPPI). Adjacent coronal sections of control and 5,7-DHT treated rat brains (n=14/group) and spinal cords (n=7/group) were labeled with each ligand and binding was measured with the use of quantitative autoradiography. (1) Levels of MPPI binding were higher in hippocampal subfields compared to PIPAT binding, whereas, in cortical layers PIPAT binding levels were higher. (2) Agonist (PIPAT) binding in hippocampal or cortical regions of 5,7-DHT treated animals was similar to controls, as has been previously observed. (3) Antagonist (MPPI) binding in hippocampal or cortical regions of 5,7-DHT treated animals was also unaltered compared to controls, except in the CA1 subfield of the hippocampus. (4) In the cervical but not the lumbar portion of the spinal cord, 5,7-DHT-treated rats showed increased levels of MPPI binding in the most superficial layer of the dorsal horn. In conclusion, the lack of changes in 5-HT$_{1A}$ agonist binding in rat brain following 5,7-DHT treatment has been extended to antagonist binding, except in the CA1 region of the hippocampus. Regional differences in the ratio of agonist to antagonist binding suggest regional differences in the coupling of 5-HT$_{1A}$ receptors to G-proteins. (Supported by MH-48125, MH-43821)

727.13

METHYLENEDIOXYMETHAMPHETAMINE INCREASES 5-HT$_{1A}$ RECEPTOR DENSITY AND mRNA EXPRESSION IN THE RAT FRONTAL CORTEX. J. Del Río*, D. Frechilla, A. García-Osta, B. Lasheras and N. Aguirre. Dept. of Pharmacology, University of Navarra, Pamplona, Spain.

Administration of 3,4-methylenedioxymethamphetamine (MDMA,"ecstasy") damages serotonergic (5-HT) axon terminals arising from the dorsal raphe. In the present study, MDMA.HCl (30 mg/kg) was given b.i.d. for 4 consecutive days to rats and the animals were sacrificed 3 h or 7 days later. As expected, 5-HT content and [^{3}H]paroxetine binding sites were markedly decreased in the frontal cortex but not in the dorsal raphe nucleus. At both survival times there was a significant increase in the density of [^{3}H]8-OH-DPAT-labelled 5-HT$_{1A}$ receptors in the frontal cortex and a concomitant decrease in receptor density in the dorsal raphe. Quantitative RT-PCR was used to measure 5-HT$_{1A}$ receptor mRNA in the two brain regions. Primers were designed to amplify a 133 bp fragment of 5-HT$_{1A}$ receptor cDNA and a 300 bp fragment of ß-actin used as the internal standard. After Southern-blot and hybridization with specific probes, an increase in 5-HT$_{1A}$ receptor mRNA expression was found in the frontal cortex of MDMA-treated rats after a 7 day survival time. It seems consequently that increased cortical receptor synthesis is promoted after lesion of 5-HT terminals. In keeping with these data, the hypothermia response to 8-OH-DPAT (1 mg/kg) was significantly potentiated 7 days after MDMA treatment. The results raise the possibility of an involvement of 5-HT$_{1A}$ receptors in the behavioral effects of MDMA. (Supported in part by SAF94-1381)

727.14

THE EFFECTS OF REDUCED pH DURING SERIAL DILUTIONS IN RADIOLIGAND COMPETITION BINDING STUDIES. W.N. Morgan, M.J. Owens, J.T. Daley, D.L. Knight, Z.N. Stowe', and C.B. Nemeroff. Dept. Psychiatry & Behav. Sci., Emory Univ. Sch. of Med., Atlanta, GA 30322.

In the performance of radioligand competition binding studies, most investigators dissolve drugs initially in ethanol, if necessary, and subsequent serial dilutions in assay buffer. Using this method, we observed competition curves that had Hill coefficients (n_H) that were much greater than unity (n_H>1.5). This also altered the apparent inhibition binding affinities (K_i). In our study, the initial use of 50% ethanol followed by serial dilutions with 5 mmol/L HCl was compared to ethanol followed by serial dilutions with assay buffer as a solvent. The former gave n_H equal to unity ($n_H \approx 1.0$) as previously predicted.

Competition binding studies were performed in triplicate on at least three separate occassions using several antidepressant drugs at the serotonin (5-HT) transporter and the 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors. [^{3}H]-citalopram, [^{3}H]-8-OH-DPAT, and [^{3}H]-ketanserin were used to label these receptor sites respectively. The antidepressants were dissolved in 50% ethanol and subsequently in either 5 mmol/L HCl or assay buffer. The 5-HT transporter K_i values (nM) in HCl and assay buffer, respectively, are: nefazodone (239; 1586), desipramine (141; 199), amitriptyline (17; 55), trazodone (258; 388), sertraline (0.32; 3), fluoxetine (2; 8), paroxetine (0.058; 0.459), venlafaxine (21; 30), and chloroimipramine (0.52; 7). At the 5-HT$_{1A}$ receptor, K_i values (nM) for HCl and assay buffer, respectively, are: nefazodone (59; 2200), desipramine (2568; 3186), amitriptyline (146; 318), trazodone (47; 185), and sertraline (4114; 7740). At the 5-HT$_{2A}$ receptor, K_i values (nM) in HCl and assay buffer, respectively, are: nefazodone (7; 2716), desipramine (161; 177), amitriptyline (5; 30), trazodone (20; 137), fluoxetine (142; 332), and paroxetine (6371; >10,000). These findings unequivocally demonstrate the need for certain drugs to be serially prepared in dilute acid to obtain curves fulfilling theoretical criteria for competitive one-site receptor-ligand interactions (i.e., $n_H \approx 1.0$).

727.15

[^{3}H]ALNIDITAN, A NEW HIGH AFFINITY AGONIST LIGAND FOR HUMAN 5HT$_{1D\alpha}$- AND HUMAN 5HT$_{1D\beta}$-RECEPTORS. J.E. Leysen*, W. Gommeren*, W.H.M.L. Luyten*, P. Vanhoenacker°, G. Haegeman°, A.S. Lesage*, *Department of Biochemical Pharmacology, Janssen Research Foundation, B-2340 Beerse, Belgium, °Laboratory of Molecular Biology, University of Gent, B-9000 Gent, Belgium.

Alniditan, (-)-(R)-*N*-[(3,4-dihydro-2*H*-1-benzopyran-2-yl)methyl]-*N'*-(1,4,5,6-tetrahydro-2-pyrimidinyl)-1,3-propanediamine dihydrochloride, is a new antimigraine agent. The compound shows high binding affinity for 5HT$_{1D}$- and 5HT$_{1A}$-receptors and weak to no binding to various other neurotransmitter or neuropeptide receptor subtypes. It is a potent constrictor of cranial blood vessels. [^{3}H]Alniditan proved to be a suitable high affinity ligand for cloned human (hu) 5HT$_{1D\alpha}$- receptors expressed in C6 glioma cells, for 5HT$_{1D\beta}$-receptors expressed in L929 cells and for 5HT$_{1D}$-receptors in calf substantia nigra tissue. The binding properties of the 5HT$_{1D}$-receptor subtypes in the three tissues were investigated using [^{3}H]alniditan in comparison with [^{3}H]5HT. [^{3}H]Alniditan revealed K_D-values (nM) of 1.25, 2.26, 1.05 and B_{max}-values (fmoles/mg protein) of 900, 1770, 750 for the receptors in the three tissues above, respectively. Investigations of binding affinities for various 5HT agonists and antagonists revealed that receptors labelled by [^{3}H]alniditan and [^{3}H]5HT were similar in the tissues, respectively. Distinctions in the binding properties of the hu 5HT$_{1D\alpha}$-, hu 5HT$_{1D\beta}$- and 5HT$_{1D}$-receptors in calf brain tissue were noted. The binding properties of the latter were more similar to those of the hu 5HT$_{1D\beta}$-receptors, although not completely identical.

Alniditan was a potent inhibitor of forskolin stimulated cAMP formation in the recombinant cells expressing the hu 5HT$_{1D\alpha}$- and hu 5HT$_{1D\beta}$-receptor, revealing full agonist properties with subnanomolar IC_{50}-values. [^{3}H]Alniditan is an advantageous ligand for both 5HT$_{1D\alpha}$- and 5HT$_{1D\beta}$-receptors, its potent agonist activities at these receptors probably underlie its antimigraine properties.

727.16

PHARMACOLOGICAL ACTIVITY OF SOLUBILIZED HUMAN 5HT1A RECEPTOR. J.T. Weber, K. Hayataka, and K. K. Parker*. Dept. of Pharm. Sci., School of Pharmacy, Univ. of Montana, Missoula, MT 59812.

The serotonin 5HT1a receptor is thought to play a role in a variety of biomedically significant disorders such as anxiety, panic disorder, and migraine. The receptor is a member of the superfamily of G protein coupled receptors, characteristically having seven transmembrane domains. The studies to be reported involve detergent solubilization of the membrane-bound receptor, analysis of agonist binding to the solubilized receptor, and probing of the receptor-G protein interface with specifically designed peptides. Solubilization of the cloned human receptor transfected into CHO cells (gift of Dr. John Raymond, Duke U.) with soldium cholate, as reported in the literature, produces receptor which binds agonist; this solubilized receptor shows nearly identical displacement of bound agonist by the partial agonist buspirone compared to non-solubilized CHO membrane receptor. CHAPS is even more effective in solubilizing activity. Agonist binding of solubilized receptor assayed with glass fiber filters has been compared to assays with G50 columns.

727.17

INTERACTIONS OF *(S)*-UH-301 DERIVATIVES WITH RECOMBINANT 5-HT$_{1A}$ RECEPTORS. Y. Gao, S. Lu, J.R. Raymond, M.N. Garnovskaya, and J.W. Kebabian*. Research Biochemicals International, Natick, MA, 01760-2447, & Duke University/VA Medical Centers, Durham, NC 27710.

(S)-UH-301 has been proposed as a specific 5-HT$_{1A}$ receptor antagonist. To test this, HeLa cell monolayers transfected with 5-HT$_{1A}$ receptor (≈2 pmol/mg of protein) were screened for alteration in the rates of H^+ efflux using a Cytosensor™ microphysiometer. Acidification rate was increased ≈45% by 5-HT, ≈35% by ±8-OH-DPAT, 40% by (+)8-OH-DPAT, 32% by (-)8-OH-DPAT, 35% by *(R)*-UH-301, and not at all by *(S)*-UH-301 (n = 3 or more for each). Those studies support a lack of agonist properties for *(S)*-UH-301. To further test that issue, ^{3}H-*(S)*-UH-301 was synthesized and saturation analyses performed. Specific binding to 5-HT$_{1A}$ receptors was ≥85%, was saturable, and of high affinity (17 ± 6 nM, n = 5). The binding was partially sensitive to 100 µM GTP, suggesting the possibility of at least some agonist properties. Several derivatives of *(S)*-UH-301 were synthesized, including two biotinylated, two affinity, and two chemical intermediate compounds. Those were tested in competition assays against ^{3}H-8-OH-DPAT using MDCK cell membranes bearing transfected 5-HT$_{1A}$ receptors (≈1 pmol/mg of protein). The results are presented below.

Compound	K_D (nM) ± SE	(n)
(S)-UH-301	20 ± 3	4
(R)-UH-301	64 ± 24	4
(S)-UH-301-biotinylated #1	96 ± 20	4
(S)-UH-301-biotinylated #2	103 ± 17	3
(S)-UH-301-photoaffinity probe	833 ± 524	5
(S)-UH-301-affinity probe	947 ± 441	4
(S)-UH-301-intermediate compound #1	63 ± 27	7
(S)-UH-301-intermediate compound #2	60 ± 23	4
p-MPPI	0.9 ± 0.3	3
WAY-100635	1.2 ± 1	3

These compounds may prove to be useful tools to study the 5-HT$_{1A}$ receptor.

727.18

WAY 100635 REVERSES THE DECREASE OF 5-HT LEVELS PRODUCED BY THE PUTATIVE 5-HT1A ANTAGONIST, WAY 100135. M.-B. Assié and W. Kock*. Neurobiology II, C.R.P.F., 17 av. Jean Moulin, 81100 Castres, France.

Using microdialysis, it was shown previously that the 5-HT1A antagonist WAY 100135, which reversed the 8-OH-DPAT-induced decrease of 5-HT levels in rat ventral hippocampus, induced, by itself, a moderate but significant decrease of 5-HT levels. The present work was aimed at characterizing this decrease using the recently described, more potent 5-HT1A antagonist WAY 100635. Chloral hydrate-anaesthetized, male Sprague Dawley rats, were implanted with a 2 mm probe in the ventral hippocampus (mm: r -4.8, l +4.6, v -7.5 according to Paxinos and Watson, 1986). The probe was perfused with artificial CSF at 1.1 µl/min. After a 2 hour stabilisation period, 20 min samples were collected and analyzed for 5-HT content by HPLC-EC. After four control samples (used to define basal level, mean = 100 %), WAY 100635 0.16 mg/kg s.c. was injected 40 min before WAY 100135 10 mg/kg s.c. or 8-OH-DPAT 0.31 mg/kg i.p., and sampling was continued for a further 140 min period. WAY 100635 produced no major changes in 5-HT levels. In some rats, however, WAY 100635 alone produced a weak and transient decrease of 5-HT levels, at 0-40 min after injection to a maximum of about 72% of the basal level. 8-OH-DPAT decreased 5-HT levels to a maximum of 36% of the basal level, and this decrease was completely abolished by pretreatment with WAY 100635. WAY 100635 also prevented the decrease induced by WAY 100135 (maximum effect: 56% of the basal level). These results are further evidence that WAY 100135 may act as a partial agonist at presynaptic 5-HT1A receptors modulating the release of 5-HT. In addition, the results suggest the possibility that the novel 5-HT1A antagonist, WAY 100635 may not be entirely devoid of intrinsic efficacy at 5-HT1A receptors.

727.19

DIRECT ACTIVATION BY DOPAMINE OF RECOMBINANT HUMAN 5-HT$_{1A}$ RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES. Murat- Oz*, Xue-Tao Li, David A. Nielsen, Forrest F. Weight and Li Zhang Lab. Molecular & Cellular Neurobiology and Lab. Neurogenetics, NIAAA, NIH, Bethesda, MD, 20892-8205.

Although an interaction between dopamine and several 5-HT receptor subtypes has been suggested, whether or not dopamine can directly activate 5-HT$_{1A}$ receptors has not been reported. In present study, cRNA of human 5-HT$_{1A}$ and the G-protein activated K^+ channel, GIRK, were coexpressed in *Xenopus* oocytes and investigated electrophysiologically. Although 5-HT, the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT, and dopamine failed to induce any detectable currents in uninjected oocytes (n=5), in oocytes expressing both 5-HT$_{1A}$ receptors and GIRK, 0.1 µM 5-HT, 1 µM 8-OH-DPAT, and 500 µM dopamine activated inward currents (n=17) that were reversibly inhibited by 0.1 µM pindobind-5-HT$_{1A}$, a 5-HT$_{1A}$ antagonist (n=5). On the other hand, a 1 µM concentration of dopamine antagonist, SCH-23390 (D_1) or spiperone (D_2), did not affect the amplitude of currents activated by 5-HT or dopamine (n=4). In addition, both 5-HT- and dopamine-induced currents had similar current-voltage relationships in the same oocytes (n=4). In oocytes coinjected with µ-opioid receptor and GIRK, even though µ-opioid agonists did not activate a current, dopamine and 5-HT did not induce detectable current suggesting that dopamine does not directly activate G-proteins or GIRK (n=5). The results suggest that dopamine can directly activate human 5-HT$_{1A}$ receptors expressed in *Xenopus* oocytes.

727.20

EFFICACY OF ANTIPSYCHOTIC DRUGS AT CLONED HUMAN SEROTONIN (5-HT)$_{1A}$ AND DOPAMINE D_4 RECEPTORS, DETERMINED BY STIMULATION OF [^{35}S]GTPγS BINDING. A. Newman-Tancredi*, V. Audinot, M. Spedding, C. Chaput L. Verrièle and M.J. Millan. Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy, Paris, France.

Dopamine D_4 and 5-HT$_{1A}$ receptors are concentrated in limbic structures associated with control of mood and emotion. We compared the ability of novel atypical antipsychotics and the neuroleptic haloperidol to stimulate [^{35}S]GTPγS binding in membranes of Chinese Hamster Ovary cells stably expressing either human 5-HT$_{1A}$ (h5-HT$_{1A}$) or D_4 (hD_4) receptors. Efficacy was defined as a percentage of the activation produced by 5-HT (10 µM) or dopamine (10 µM), respectively (= 100%). Affinity (K_i) values were determined in competition binding experiments with [^{3}H](±)8-OH-DPAT (h5-HT$_{1A}$) or [^{3}H]spiperone (hD_4). Drugs were tested at concentrations of 0.1 nM to 10 µM.

	h5-HT$_{1A}$		hD_4	
Antipsychotic	**Ki (nM)**	**Efficacy (%)**	**Ki (nM)**	**Efficacy (%)**
Haloperidol	**2148**	**0**	**1.9**	**0**
Clozapine	**160**	**59**	**35.5**	**0**
S 16924	**1.9**	**66**	**6.2**	**0**
S 17828	**169**	**0**	**2.0**	**0**

Whereas haloperidol and S 17828 acted as (low affinity) antagonists at h5-HT$_{1A}$ receptors, clozapine and S 16924 had partial agonist activity at this site. S 16924 had the highest affinity. All compounds showed significant affinity, but low intrinsic activity, at hD_4 receptors. These interactions of antipsychotic drugs at h5-HT$_{1A}$ and hD_4 receptors may be of importance for their therapeutic properties.

727.21

[^{3}H]S 15535, A NOVEL, SELECTIVE RADIOLIGAND AT SEROTONIN (5-HT)$_{1A}$ RECEPTORS: CHARACTERISATION OF BINDING TO CLONED HUMAN AND RAT HIPPOCAMPAL 5-HT$_{1A}$ RECEPTORS. J-L. Peglion*, A. Newman-Tancredi, L. Verrièle and M.J. Millan, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy, Paris, France.

The novel benzodioxopiperazine, S 15535 (4-(benzodioxan-5-yl)1-(indan-2-yl)piperazine) is a highly selective ligand for serotonin 5-HT$_{1A}$ receptors (Millan et al, J.P.E.T., 268, 337-352, 1994). The compound was tritiated (8 Ci/mmol) and its binding profile characterised in CHO cells stably expressing the human 5-HT$_{1A}$ (h5-HT$_{1A}$) receptor. At 22 °C, [^{3}H]S 15535 associated to h5-HT$_{1A}$ receptors with a half-time of 3.4 minutes. In saturation binding experiments, [^{3}H]S 15535 displayed a K_d (1.3 nM) and a B_{max} (1.4 pmol/mg) similar to that observed in this cell line with the prototypic 5-HT$_{1A}$ agonist, [^{3}H](±)-8-OH-DPAT (1.0 nM and 1.1 pmol/mg). The K_d for S 15535 agreed well with the K_i value derived from competition of unlabelled S 15535 with [^{3}H]8-OH-DPAT (0.8 nM). In competition binding experiments with [^{3}H]S 15535, serotonergic ligands, including (±)-8-OH-DPAT, 5-HT, (+)-WAY 100,135 and spiperone, displayed K_is consistent with those observed with [^{3}H](±)-8-OH-DPAT, with values of 1.2, 1.7, 14 and 460 nM respectively. GppNHp (100 µM) only slightly reduced the binding of S 15535 (- 18 %) as compared to that of [^{3}H]8-OH-DPAT (- 70 %) to h5-HT$_{1A}$ receptors. Further, ATP (100 µM) also inhibited (- 10 %) [^{3}H]S 15535 binding. [^{3}H]S 15535 showed high affinity, saturable binding to rat hippocampal membranes (K_d = 2.4 nM; B_{max} = 820 fmol/mg) although its association rate was slower (half-time = 7.3 min). As for h5-HT$_{1A}$ receptors, the binding of [^{3}H]S 15535 to hippocampal membranes was slightly reduced (- 10 %) by GppNHp (100 µM). These results suggest that [^{3}H]S 15535 exerts antagonist (or weak partial agonist) properties at both human and rat 5-HT$_{1A}$ receptors for which it represents a novel, useful radioligand.

727.22

EFFECTS OF 5-HT$_{1A}$ AND 5-HT$_{1B}$ AGONISTS ON VENTRAL PALLIDAL NEURONAL ACTIVITY. **B.A. Heidenreich*, F. Rehman & T.C. Napier. Dept. Pharmacol., Stritch Sch. Med., Loyola Univ. Chicago, Maywood, IL 60153.**

The ventral pallidum (VP) of the basal forebrain contains high levels of serotonin (5-HT). In addition, 5-HT$_{1A}$ receptors exist on the somata of VP neurons and 5-HT$_{1B}$ receptors are found on the pallidal terminals of striatal projection neurons. The present study compared the effects of the 5-HT$_{1A}$ agonist (±)8-OH-DPAT (1-256 µg/kg i.v.) and the 5-HT$_{1B}$ agonist CP-94253 (0.01-6.4 mg/kg i.v.) with the non-selective 5-HT agonist TFMPP (0.01-2.9 mg/kg i.v.) on single-neuron activity in the VP of chloral hydrate-anesthetized rats. TFMPP dose-dependently reduced the firing rate of 100% of VP neurons tested (n = 7). In contrast, 8-OH-DPAT produced dose-dependent increases (n = 7) and decreases (n = 4) in VP activity. CP-94253 had no effect on 5 VP neurons and produced only transient and/or variable changes in the activity of 7 additional neurons. Within the latter group, 3 neurons decreased firing rate but these responses were not antagonized by the 5-HT$_{1A/1B}$ antagonist (-)pindolol (0.1-0.2 mg/kg). These results suggest that the alterations in VP neuronal activity produced by TFMPP were not mediated by 5-HT$_{1B}$ receptors. While 5-HT$_{1A}$ receptor activation decreased the firing rate of some VP neurons, other 5-HT receptor subtypes are also likely to contribute to the effects of the TFMPP on VP activity.

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727.23

SEROTONIN INHIBITS NUCLEUS ACCUMBENS NEURONS RECEIVING INPUTS FROM PARAFASCICULAR NUCLEUS OF THALAMUS VIA 5-HT1A RECEPTORS BUT NOT THOSE FROM HIPPOCAMPUS. I. NAGAOKA*[1], A. TAKU[2], M. SASA[2], S. YAMAWAKI[1]. Departments of [1]Psychiatry and Neurosciences and [2]Pharmacology Hiroshima University School of Medicine, Minami-ku, Hiroshima, 734, Japan.

The nucleus accumbens (Acc) neurons receives innervation from various areas of the brain such as parafascicular nucleus (Pf), hippocampus, amygdala and ventral tegmental area. Serotonin (5-HT) nerve terminals and 5-HT receptor subtypes (5-HT1A, 5-HT1B, 5-HT1C, 5-HT2 and 5-HT3 receptors) are found in Acc. However, the role of 5-HT receptors in Acc neurons functionally remains unknown. Therefore, microiontophoretic studies were performed to elucidate the role of 5-HT1A receptors of Acc neurons receiving inputs from Pf and hippocampus (HPC). Single neuronal activities were recorded with a glass microelectrode attached along a seven-barreled micropipet in chloral hydrate-anesthetized rats. Each of the barrels was filled with dopamine (DA), 5-HT, 8-OH-DPAT(5-HT1A agonist), NAN-190 (5-HT1A antagonist), noradrenaline, glutamate and 3M NaCl, accordingly. Drugs were applied to the immediate vicinity of the target neuron recorded using by microiontophoresis method. Spikes elicited by the Pf stimulation were inhibited by iontophoretically applied DA or 5-HT and 8-OH-DPAT in a dose-dependent manner. Glutamate-induced firing was also inhibited by simaltaneous application of DA, 5-HT or 8-OH-DPAT. 8-OH-DPAT induced inhibition of glutamate-induced firing was antagonized drug application of NAN-190. However, spikes elicited by HPC stimulation were not affected by iontophoretically applied DA or 5-HT. These results suggest that the DA-sensitive Acc neurons receiving inputs from Pf are inhibited by 5-HT via 5-HT1A receptors but those from hippocampus are not affected.

727.24

EFFECTS OF 8-OH-DPAT ON RELEASE OF CORTICOTROPIN RELEASING FACTOR IN AMYGDALA. E.A. Johnson*, V. Garlapati and D.L. Birkle. Dept.s of Behavioral Medicine and Psychiatry, Neurology, Pharmacology and Toxicology, West Virginia University. School of Medicine, Morgantown, WV 26506.

Corticotropin Releasing Factor (CRF) is a 41 amino acid peptide neurotransmitter with putative roles in stress and anxiety. Release of CRF from the hypothalamus results in increased release of ACTH from the anterior pituitary, ultimately leading to glucocorticoid release in the stress response. Increasing evidence indicates that release of CRF from sites in the central nervous system, other than the hypothalamus, may play a role in anxiety. The amygdala is a brain region rich in CRF neuronal terminals. Furthermore, the amygdala functions as a modulator of anxiety. The amygdala is innervated by serotonergic neurons originating in the dorsal and medial raphe nuclei. Amygdala contains a moderate level of 5-HT$_{1A}$ receptors. Since 8-OH-DPAT, a selective agonist of 5-HT$_{1A}$ receptors, has been demonstrated to stimulate CRF release in hypothalamus, we investigated its effect on CRF release from amygdala. Amygdala minces were incubated *in vitro* with 8-OH-DPAT (0.01-100 nM) under basal and depolarizing conditions. 8-OH-DPAT caused no change in basal CRF release but dose dependently increased potassium-induced release of CRF from amygdala minces ($p < 0.05$). The maximal effect was observed using 1 nM 8-OH-DPAT which is consistent with the affinity reported for 8-OH-DPAT binding to 5-HT$_{1A}$ receptors (0.7-2.0 nM). These results are consistent with previous reports of the stimulatory action of 8-OH-DPAT on hypothalamic release of CRF.

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727.25

AGONIST-INDUCED [^{35}S]GTPγS BINDING AT HUMAN 5-HT$_{1A}$ RECEPTORS STABLY EXPRESSED IN HeLa CELLS

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In this study the pharmacological characterisation of agonist-induced [^{35}S]GTPγS binding to the human 5-HT$_{1A}$ receptors stably expressed in HeLa cells (HA7) was investigated. This response was also used to investigate the presence of receptor reserve in this cell line.

HeLa cells expressing human 5-HT$_{1A}$ receptors (5mg wet weight/tube) were labelled with 100pM [^{35}S]GTPγS in the presence of 30µM GDP (Lazareno and Birdsall, 1993). Pretreatment of these cells with 3µM, 6µM and 10µM benextramine, resulted in reduced 5-HT maximal response and potency. Furchgott analysis (1966) yielded a pK_A 6.98±0.03 compared with a pK_{obs} of 8.4±0.3, obtained from [^{3}H]5-HT radioligand binding saturation studies, indicating the presence of receptors in mixed affinity states. Plots of receptor occupancy verses maximal response indicate the presence of some receptor reserve. In unpretreated cells 5-HT, 5-CT, 8-OH DPAT, RU24969, and buspirone displayed full and NAN 190 partial agonism. WAY 100135 had no intrinsic activity whereas methiothepin dose dependently reduced basal levels.

This method offers an efficient means of assessing the potency and efficacy of compounds at the human cloned 5-HT$_{1A}$ receptor.

Furchgott, R.F. (1966) Advances in drug research, 3, 21-55.
Lazareno, S. and Birdsall, N.J.M. (1993) Br.J.Pharmacol., 109, 1120-1127.

728.1

THE 5-HT5 RECEPTORS: CHARACTERIZATION OF THE HUMAN 5-HT5A RECEPTOR; ABSENCE OF THE HUMAN 5HT5B RECEPTOR; KNOCKOUT OF THE MOUSE 5HT5A RECEPTOR. R. Grailhe, S. Ramboz, U. Boschert+ and R. Hen*. Center for Neurobiology and Behavior, Columbia University, New York, NY 10032, USA. +Glaxo Institute for Molecular Biology, Geneva, Switzerland.

We have isolated from a human genomic library, the homologues of the mouse 5-HT5A and 5-HT5B genes. The human 5-HT5A gene encodes a protein with similar characteristics as the mouse 5-HT5A receptor. When expressed in Cos-7 cells, the human 5-HT5A receptor displayed a high affinity for the radiolabelled ligands [^{3}H]5-CT (Kd=2,8nM) and [^{125}I]LSD (Kd=187pM). These sites were insensitive to GppNHp and displayed a similar pharmacological profile as the mouse 5HT5 receptors. We are currently analyzing the coupling of the human 5-HT5A receptor in NIH3T3, 293 and GH3 cells. Reverse PCR experiments revealed expression of the human 5-HT5A mRNA in cortex, hyppocampus, amygdala, thalamus, raphe nuclei and spinal cord .

The human 5-HT5B gene is composed of two exons and is transcribed at very low level. However, unlike the 5-HT5A gene, the 5-HT5B gene does not appear to encode a functional protein, since the putative coding sequence is interrupted by several stop codons. In addition, Southern and PCR experiments performed on samples from different individuals, have revealed that this non-functional gene is the only human homologue of the rodent 5HT5B gene. These results indicate that humans do not express a functional 5-HT5B receptor and suggest that this protein has been lost after the divergence between rodents and primates.

In order to study the function of the 5-HT5A receptor, we have generated by homologous recombination, mutant mice lacking the gene encoding this receptor. These homozygous mutant mice develop, move, feed and breed apparently normally. We are currently investigating their behavior in various experimental paradigms as well as their response to drugs that have a high affinity for the 5-HT5A receptor, such as LSD.

728.2

THE 5-HT$_{5A}$ SEROTONIN RECEPTOR IS EXPRESSED PREDOMINANTLY ON ASTROCYTES WITHIN THE DEVELOPING AND ADULT RAT CNS. M. J. Carson*, P. E. Danielson, E. A. Thomas, and J. G. Sutcliffe. The Scripps Research Institute, La Jolla, CA 92037

Serotonin (5-hydroxytryptamine, 5-HT) elicits a wide array of sensory, motor, and behavioral processes in the mammalian central nervous system (CNS). Identification and characterization of the individual receptors which mediate these responses is crucial to understanding the mechanisms of serotonin's actions within the CNS. Using antisera to two synthetic peptides corresponding to non-overlapping regions of the rat 5-HT$_{5A}$ receptor, we detected primarily astrocytes in immunocytochemical analysis. Throughout all time points examined (P1 through adulthood), 5-HT$_{5A}$ immunoreactivity colocalized with the astrocyte specific marker glial fibrillary acidic protein. 5-HT$_{5A}$ immunoreactivity was present on both the cell body and the stellate processes of the astrocyte. RT-PCR analysis confirmed the expression of 5-HT$_{5A}$ in neuron-depleted astrocyte cultures. By northern blot analysis, 5-HT$_{5A}$ expression was detected as early as E18 in rat CNS and peaked by P20 declining only slightly in adulthood. Together, these results indicate a mechanism for glial-neuronal serotonergic interactions which develops *in vivo* in concert with astrocyte development. To understand the consequences of these interactions, we are comparing the signal transduction events mediated by 5-HT$_{5A}$ in glial cell lines naturally expressing 5-HT$_{5A}$ with non-glial cell lines transfected with 5-HT$_{5A}$.

728.3

5-HT$_4$ RECEPTORS IN RAT BRAIN: mRNA AND RECEPTOR DISTRIBUTIONS SUGGEST BOTH SOMATODENDRITIC AND PRESYNAPTIC LOCALIZATIONS. M.T.Vilaró[1], G.Mengod[1], R.Cortés[1], C.Gerald[2], T.A.Branchek[2], and J.M.Palacios[3]* 1:Dpt.Neurochem. CID/CSIC, Barcelona 08034, Spain; 2:Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

Oligonucleotide probes and *in situ* hybridization were used to study the distribution of mRNA encoding the long (5-HT$_{4L}$) and short (5-HT$_{4S}$) splice variants of the serotonin 5-HT$_4$ receptor. With a probe common to both variants, the strongest hybridization signals were detected in olfactory tubercle (OTu), islands of Calleja (ICj) and medial habenula (MHb). Strong signals were observed in nucleus accumbens (Acc) and caudate-putamen (CPu), this latter showing a rostro-caudal increasing gradient of signal. Intermediate and low signals were seen, respectively, in the granule cell layer of the dentate gyrus and in the pyramidal cell layer of Ammon's horn. With a probe specific for 5-HT$_{4L}$, essentially the same pattern was observed. Finally, a probe specific for 5-HT$_{4S}$ presented a more restricted pattern of distribution, with low levels of signal in OTu, ICj, MHb, Acc and caudal parts of CPu and very low levels in dentate gyrus.

5-HT$_4$ receptor binding sites were visualized with the 5-HT$_4$ antagonist [^{125}I]SB 207710. The highest densities of receptors were observed in OTu, Acc, CPu, globus pallidus (GP), substantia nigra (SN) and interpeduncular nucleus (IP). Lower densities were present in medial and lateral habenula and hippocampus, among many others. Comparison of mRNA and receptor distributions suggests two different subcellular localizations for 5-HT$_4$ receptors. Receptors present e.g. in OTu, Acc, CPu and hippocampus would be located somatodendritically, whereas receptors present e.g. in GP, SN and IP would be located pre-synaptically on axon terminals of, respectively, the striatopallidal, striatonigral, and habenulo-interpeduncular projections.

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728.4

5-HYDROXYTRYPTAMINE 4 RECEPTORS (5-HT$_4$R) ARE LOCATED PRESYNAPTICALLY IN THE STRIATONIGRAL PATHWAY AND CODISTRIBUTED WITH 5-HT$_{1D}$ RECEPTORS. G.Mengod[1*], R. Cortés[1], C. Salcedo[2], and J.M. Palacios[2]. [1]Dept. Neurochem., CID/CSIC, 08034 Barcelona, Spain.

The localization of 5-HT$_4$ receptors in the basal ganglia of guinea-pig has been studied using autoradiographic techniques and ^{125}I-SB 207710 as a ligand. In consecutive sections, 5-HT$_{1D}$ receptors were visualized using ^{125}I-GTI. Caudate-putamen, globus pallidus and substantia nigra pars reticulata were some of the areas presenting the highest densities of receptors. In order to examine the possible cellular localization of these receptors, neurotoxic lesions were performed. Dopamine neurons were destroyed by direct injection of 6-OHDA into the substantia nigra. In animals bearing these lesions no modification on the distribution or density of 5-HT$_4$ and 5-HT$_{1D}$ receptors were measured. In contrast, lesion of the intrinsic neurons of the caudate-putamen nucleus with quinolinic acid, resulted in a marked reduction of receptor densities in areas of the caudate-putamen surrounding the lesion placement and also in limited regions of the ipsilateral substantia nigra pars reticulata. The localization and extension of 5-HT$_4$ receptor losses coincided with those of 5-HT$_{1D}$ sites. These results suggest that 5-HT$_4$ in the guinea-pig basal ganglia are not located in dopaminergic neurons but rather on neurons intrinsic to the striatum which project to the substantia nigra such as those utilizing substance P, enkephalin and GABA as neurotransmitters, where these sites are codistributed with 5-HT$_{1D}$ receptors.

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728.5

5-HT$_4$ RECEPTOR LEVELS ARE REDUCED IN THE PUTAMEN FROM PATIENTS WITH SCHIZOPHRENIA.
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Relatively high densities of the 5-HT$_4$ receptors are expressed in human mesolimbic and nigro-striatal systems, as detected using the high affinity and selective radioligand [^{3}H]GR113808. Our recent studies in rat brain indicate that the 5-HT$_4$ receptor can modulate the release of dopamine. It is generally accepted that an overactivity of the mesolimbic dopamine system is associated with schizophrenia. Therefore, in the present studies we investigated the levels of 5-HT$_4$ receptors in human brain regions from schizophrenic patients and matched control patients who had died without a neurological or psychological disorder.

Saturation studies with [^{3}H]GR113808, revealed specific binding (non-specific defined by SDZ 205-557, 10 μM) in all examined tissues from control and schizophrenic patients (putamen, amygdala, nucleus accumbens and substantia nigra). There was a significant ($p < 0.05$) reduction in [^{3}H]GR113808-labelled 5-HT$_4$ receptor density in putamen tissue from schizophrenic patients when compared to control putamen tissue (approximately 50 %). Whilst no significant differences in the densities of the radiolabelled 5-HT$_4$ receptor were detected in amygdala, nucleus accumbens and substantia nigra from schizophrenic and control patients. Whether the reduction in 5-HT$_4$ receptor density in the putamen of schizophrenic patients, is part of the pathology of the psychiatric disorder or a consequence of chronic psychotropic drug therapy, remains to be established. We would like to thank the MRC Brain Bank (Cambridge, UK) for the donation of tissue.

728.6

ABILITY OF THE 5-HYDROXYTRYPTAMINE$_4$ RECEPTOR TO MODULATE DOPAMINE AND 5-HYDROXYTRYPTAMINE RELEASE IN RAT BRAIN

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In the present study we assess the ability of the 5-hydroxytryptamine$_4$ (5-HT$_4$) receptor to modulate dopamine (DA) and 5-hydroxytrptamine (5-HT) release in the rat forebrain.

The 5-HT$_4$ receptor agonist renzapride enhanced dopamine release from both rat striatal slices and in the striatum of freely moving rats assessed by the microdialysis technique in a concentration dependent manner (maximal increase; 214±35% in slices (10 μM renzapride) and 161±10% in vivo (100 μM renzapride administered via the probe, mean±SEM, n=4-14). The renzapride-induced increase in DA release (both preparations) was prevented by the selective 5-HT$_4$ receptor antagonist, GR113808 (in vitro 100 nM , in vivo 1 μM) and the protein kinase A inhibitor, H7 (in vitro 100 nM, in vivo 1 μM). Similar responses were detected with the 5-HT$_4$ receptor agonist 5-methoxytryptamine (5-MeOT).

The 5-HT$_4$ receptor agonists 5-MeOT (10 μM; with concomitant 5-HT$_1$/5-HT$_2$ receptor blockade) and renzapride (100 μM), maximally increased 5-HT release in the hippocampus, assessed by the microdialysis technique, by 451±40% and 214±30%, respectively (mean±S.E.M., n=3-5). Lower concentrations of renzapride (1-10 μM) were less effective. GR118303 (1 μM), reduced dialysate levels of 5-HT (maximally 50±8%, mean±S.E.M., n=4) and also completely antagonised the increase in 5-HT release induced by either 5-MeOT (10 μM) or renzapride (100 μM).

The present studies indicate that the 5-HT$_4$ receptor modulates DA and 5-HT release in the striatum and hippocampus, respectively.

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728.7

CO-LOCALIZATION OF SEROTONIN 5-HT6 AND 5-HT2C RECEPTORS IN NEUROPEPTIDE CONTAINING NEURONS OF THE RAT STRIATUM

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The striatum is the largest nucleus of the basal ganglia, and has two main outputs: a projection to the globus pallidus, and a projection to the substantia nigra. Dopamine receptors and certain neuropeptides which are expressed in the striatum are differentially distributed between these two pathways. Specifically, enkephalin and the dopamine D2 receptor are expressed in neurons projecting to the globus pallidus, while substance P and dynorphin along with the dopamine D1 receptor are expressed in neurons projecting to the substantia nigra. The striatum also receives a prominent serotonergic input, but little is known about how serotonin receptors fit into the molecular neuroanatomy described above. In the present study we used double label *in situ* hybridization to determine the distribution of the mRNAs of two of the serotonin receptors, the 5-HT6 and the 5-HT2C, in relation to enkephalin, substance P, and dynorphin expressing output neurons.

Coronal sections of rat brain were simultaneously hybridized with an ^{35}S riboprobe for one of the serotonin receptors and a digoxygenin labeled riboprobe for one of the neuropeptides. Sections were examined using brightfield microscopy, and the relationship of expression of the two mRNAs to each other was determined.

mRNA for the 5-HT6 receptor was found to be evenly distributed throughout the striatum and nucleus accumbens and to co-localize with all three of the neuropeptides examined. This indicates that signals mediated via this receptor will affect both of the major striatal output pathways.

The 5-HT2C receptor also showed co-localization with all three of the neuropeptides examined, however its distribution is considerably more complex. Levels of 5HT-2C mRNA were much higher medially than laterally, and ventrally than dorsally. Additionally discreet regions found throughout the striatum exhibited much higher levels of expression. These small scattered regions represent the striatal patch compartment, as shown by their overlap with regions which exhibit high levels of dynorphin mRNA expression. Supported by NS20311 and a P.M.A.F. Fellowship.

728.8

AFFINITY OF TYPICAL AND ATYPICAL NEUROLEPTICS FOR THE CLONED HUMAN 5-HT$_6$ SEROTONIN RECEPTOR

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The 5-HT$_6$ receptor is a G_s coupled serotonin receptor with relatively low sequence homology to other known serotonin receptor subtypes. One of the most striking features initially described for the rat 5-HT$_6$ receptor was its high affinity for the atypical neuroleptic clozapine and related compounds. This affinity and its distribution in brain areas such as striatum, nucleus accumbens and hippocampus thought to be involved in neuroleptic drug action suggested that the 5-HT$_6$ receptor might be important in mediating some of the advantageous differences of atypical over typical antipsychotic drugs.

We have now extended the studies on neuroleptic affinity for the rat 5-HT$_6$ receptor by Roth and co-workers to the cloned human 5-HT$_6$ receptor expressed in COS-7 cells. It also has high (approximately 10 nM) affinity for several atypical neuroleptics including clozapine, olanzepine, ICI 169369, rilapine and (-)octoclothepin. Although chlorpromazine and (+)octoclothepin, both typical neuroleptics, also have high 5-HT$_6$ affinity, affinities for most typical neuroleptics is lower, as are those of atypical drugs with some residual liability to produce extrapyramidal symptoms. These data suggest that 5-HT$_6$ receptors, along with others such as 5-HT$_{2A}$, may play an important role in mediating some of the differences between typical and atypical antipsychotic drugs. *Supported by the Department of Veterans Affairs.*

728.9

EFFECTS OF CLOZAPINE AND HALOPERIDOL ON EXPRESSION OF 5HT$_6$ AND 5HT$_7$ RECEPTORS

J.A. Frederick, J.F. Lopez*, and J.H. Meador-Woodruff. Mental Health Research Institute, Dept. of Psychiatry, University of Michigan, Ann Arbor, MI 48109. The distribution and regulation of messenger RNA encoding two recently cloned serotonin receptors was examined by in situ hybridization in the rat brain. 5-HT$_6$ labelling was observed in the striatum, olfactory tubercle, neocortex, and hippocampus. 5-HT$_7$ labelling was observed in the thalamus, hypothalamus, piriform cortex, entorhinal cortex, superficial layers of neocortex, septum, amygdyla, and the CA2 and CA3 regions of the hippocampus. Most striking was the lack of 5-HT$_7$ expression in the dentate gyrus or CA1 regions of hippocampus, or in the striatum. The high affinity of 5-HT$_6$ and 5-HT$_7$ receptors for atypical antipsychotic drugs, and their localization in limbic and cortical regions, suggests that they may play a role in the pathophysiology of schizophrenia. To determine if expression of these receptors is regulated by antipsychotic drugs, rats were injected with clozapine (20 mg/kg/day, N=8), haloperidol (2 mg/kg/day, N=8), or vehicle (N=8) for two weeks. Preliminary evidence suggests that clozapine and haloperidol increase expression of 5-HT$_6$ receptors in the nucleus accumbens, and decrease 5-HT$_6$ expression in frontal cortex. Meanwhile, no differences in 5-HT$_6$ expression were observed between haloperidol- and clozapine-treated animals in all regions studied. Further results of this study are expected and will be presented.

728.10

CORRELATION BETWEEN 5-HT$_7$ RECEPTOR BINDING AND ITS mRNA IN THE RAT AND GUINEA PIG BRAIN VISUALIZED AUTORADIOGRAPHICALLY.

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We have used receptor autoradiography with ^{3}H-5-carboxytryptamine (^{3}H-5-CT) as ligand and *in situ* hybridization histochemistry using ^{32}P-labeled oligonucleotides to visualize the anatomical localization of the 5-HT$_7$ receptor and its mRNA in the rat and guinea pig brain.

By analyzing the displacement of 5 nM ^{3}H-5-CT binding by the selective 5-HT$_{1A}$ and 5-HT$_{1D}$ antagonists WAY 100135 and GR 127935 and 8-OH-DPAT, a 5-HT$_{1A}$ and 5-HT$_7$ ligand, conditions were selected allowing for the direct visualization of 5-HT$_7$ binding sites. In the guinea pig, 5-HT$_7$ receptors could be selectively labeled with 5 nM ^{3}H-5-CT in the presence of high concentrations of WAY 100135 (100 μM) and GR 127935 (200 μM), whereas in the rat, a small population of 5-HT$_{1B}$ receptors remained still labeled.

In these conditions, in the guinea pig 5-HT$_7$ binding sites were found to be enriched in layers I-III of the neocortex, claustrum, several hypothalamic and midline nuclei of the thalamus (i.e paraventricular, paratenial, mediodorsal nuclei), dentate gyrus, and superior colliculus, among others. These areas were also enriched in 5-HT$_7$ mRNA signal, both in rat and guinea pig. In contrast, neither 5-HT$_7$ binding nor mRNA were detected in structures such as the globus pallidus or the pars compacta and reticulata of the substantia nigra.

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OTHER NEUROTRANSMITTERS: HISTAMINE

729.1

[3H]-THIOPERAMIDE-A NEW RADIOLIGAND FOR THE HISTAMINE H3 RECEPTOR

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Thioperamide is the first selective high affinity antagonist described for the H3 receptor. Its use as a radioligand has, sofar, never been reported. In the present study, the binding of [3H]-thioperamide was characterized in membranes of rat brain cortex. Transformation of saturation curves showed non-linear Scatchard plots suggesting labelling of two different populations of sites by [3H]-thioperamide. Up to a concentration of 1 µM, H3 agonists hardly displaced the total binding of 5 nM [3H]-thioperamide. Moreover, although H3 antagonists fully displaced the total binding of 5 nM [3H]-thioperamide Ki's obtained were different from the values expected for the H3 receptor. These results showed that, at 5 nM, [3H]-thioperamide was mainly labelling a non-H3 receptor site. The total binding of 0.3 nM [3H]-thioperamide, however, was, inhibited up to 60% by H3 agonists. Stereoselectivity for the R and S isomers of a-methylhistamine and guanine nucleotides sensitivity for the displacement by R-a-methylhistamine was established. H3 antagonists fully displaced the binding of 0.3 nM [3H]-thioperamide. Displacement curves of the antagonists were biphasic with the Ki for the high affinity site similar to values expected for the H3 receptor. In conclusion, in rat brain, [3H]-thioperamide, labels with high affinity both the H3 receptor and a indentified non-H3 binding site. Most of the H3 antagonists also show high affinity for the non-H3 site. Because agonists do not show high affinity for the non-H3 component of the binding of [3H]-thioperamide, they should be used to define the nonspecific binding of [3H]-thioperamide when studying the H3 receptor.

729.2

OXIDATION OF HISTAMINE IN RODENT BRAIN HOMOGENATES: EVIDENCE FOR AN ALTERNATIVE PATHWAY OF BRAIN HISTAMINE METABOLISM.

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There is extensive evidence from early studies that histamine is mainly, if not exclusively methylated in mammalian brain. However, we recently showed that imidazoleacetic acid (IAA), histamine's oxidative metabolite in the periphery and a potent GABA$_A$ agonist, is present in brain where its levels increase when histamine methylation is blocked. IAA does not enter brain from periphery. ^{3}H-histamine (icv) formed ^{3}H-IAA and its ribosylated metabolites in rat brain, a process that increased when histamine methylation was blocked (*J. Neurochem.* **65** [in press]). To assess which enzyme(s) may be involved, we examined metabolism of 2-10 ng ^{3}H-histamine in whole brain homgenates of male rats, mice and guinea pigs (2 ml; 5 vol; NaP buffer, pH 7.4) incubated (37°C) with amine oxidase inhibitors (e.g. clorgyline, deprenyl or aminoguanidine [AG]), then boiled after 30 min. Aliquots (5 µl) were applied to Polygram silica TLC plates (250 µ); t-Butanol, formatic acid, water (70,15,15) separated histamine (R_F 0.31), *tele*-methylhistamine (0.14), *tele*-methylimidazoleacetic acid (0.50) and IAA and its metabolites (0.63-0.66). In all species examined, ^{3}H-IAA (and/or its metabolites) was produced. Clorgyline and deprenyl, at concs selective for MAO-A and -B, respectively, did not affect recoveries of ^{3}H-histamine or ^{3}H-IAA (and its metabolites). In contrast, at concs expected to abolish DAO activity (up to 1 µM), AG inhibited, but did not abolish oxidation. These results confirm that histamine is not oxidized by MAO-A or -B and suggest that an AG-sensitive enzyme, possibly DAO or another amine oxidase(s), can oxidize histamine in brain and form IAA and its metabolites. (NINDS-NS-28012)

729.3

COMPARISON OF HISTAMINE H_3 RECEPTOR ANTAGONISTS (GT-2016, THIOPERAMIDE, AND CLOBENPROPIT): RECEPTOR BINDING AND HISTAMINE TURNOVER. C.E. Tedford*, J.G. Phillips, M.A. Khan, R.C.A. Frederickson, R. Leurs**, H. Timmerman**, and S.L. Yates. Gliatech Inc., 23420 Commerce Park Rd. Cleveland, OH 44122 and **Vrije Universteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

The release of histamine (HA) in the CNS is under the negative tonic control of presynaptic HA H_3 autoreceptors. Once released, HA is metabolized to N^{π}-methylhistamine (TMH). Selective H_3 receptor antagonists block the negative feedback of HA, resulting in increased HA release and metabolism. GT-2016, a nonthiourea-containing H_3 antagonist, has been developed at Gliatech. *In vivo* CNS penetration of GT-2016 and changes in HA turnover were studied in male Sprague Dawley rats, and compared to the thiourea- and isothiourea-containing H_3 antagonists, thioperamide and clobenpropit. The *in vitro* K_i's for GT-2016, thioperamide, and clobenpropit were 44, 1.6, and 0.2 nM, respectively, using rat cortical membranes and 1 nM ^{3}[H]N^{α}-methylhistamine (NAMHA). The ability of these compounds to penetrate the CNS was assessed using an *ex vivo* binding technique. Rats were pretreated with drug (1 hr) and an inhibition binding assay was performed using unwashed cortical homogenates. Each of the drugs (1 to 30 mg/kg; ip) produced a dose-dependent inhibition of NAMHA binding. The CNS bioavailability of GT-2016 was 10 to 15 fold greater than that of thioperamide and approximately 150 fold greater than the CNS bioavailability of clobenpropit. Clobenpropit produced minimal changes in HA and TMH levels. GT-2016 and thioperamide produced dose-dependent increases in the TMH/HA ratio, indicative of increased HA release and metabolism. These increases in the TMH/HA ratios were directly correlative with the degree of receptor binding shown in the *ex vivo* binding studies. In summary, GT-2016, a novel nonthiourea H_3 antagonist, provides good CNS bioavailability and corresponding functional blockade of CNS H_3 receptors.

729.4

HISTAMINE AGONISTS AND ANTAGONISTS: RECEPTOR SUBTYPE AFFINITIES AND SELECTIVITIES. N.A.Sharif* and S.Xu. Molecular Pharmacology Unit, Alcon Laboratories Inc., Fort Worth, TX 76134.

Specific [^{3}H]pyrilamine, [^{3}H]tiotidine and [^{3}H]N-methyl histamine binding to rodent brain H_1, H_2 and H_3 histamine receptors was studied. Drugs with high affinity (K_i = 0.8-3 nM) for H_1 sites included emedastine (a novel ocular anti-histamine), triprolidine, pyrilamine and ketotifen. Ranitidine showed the highest affinity for H_2 sites (K_i = 187 nM). Drugs with high affinity (K_i = 0.2-5 nM) for H_3 sites included imetit, thioperamide and histamine. Emedastine showed the highest H_1-selectivity (37,000-fold) relative to its H_2-affinity. Ranitidine was the most H_2-selective, and thioperamide (254,000-fold) and imetit (240,000-fold) the most H_3-selective relative to their H_1-affinities. Histamine stimulated phosphoinositide (PI) turnover in human corneal fibroblasts (EC_{50} = 1 μM), and the H_1-antagonists, emedastine (IC_{50} = 0.6 nM), levocabastine (IC_{50} = 11 nM) and triprolidine (IC_{50} = 4 nM) potently inhibited the histamine PI responses. These potencies matched their H_1-receptor binding affinities. These data have shown emedastine to be an high-affinity and high potency histamine antagonist with the highest H_1-selectivity. Emedastine may therefore be a useful H_1-antagonist for use in the eye to treat allergic diseases and in the brain for affective and other disorders.

729.5

Effects of Histamine on Phospholipase C and Adenylyl Cyclase Activities in Porcine Spinal Arachnoid Cells in Culture. Martin T. Taylor* and Edward L. Orr, Department of Anatomy and Cell Biology, U.N.T. Health Science Center, Fort Worth, TX 76107

Structural and biochemical interactions between arachnoid cells form the basis of the cerebrospinal fluid (CSF)-blood barrier. Using cultured porcine spinal arachnoid cells, we have begun to assess the types of receptor/second messenger systems expressed by arachnoid cells that may be involved in regulating the CSF-blood barrier. Activation of phospholipase C (PLC) was measured in confluent arachnoid cells by monitoring the conversion of ^{3}H-myoinositol to ^{3}H-inositol phosphates. Adenylyl cyclase (AC) activity was measured by preincubating the cells in ^{3}H-adenine to form ^{3}H-ATP then measuring the conversion of ^{3}H-ATP into ^{3}H-cyclic AMP. Porcine spinal arachnoid cells *in vitro* expressed both PLC and AC activity. Specifically, both carbachol and histamine stimulated PLC at EC_{50}s of 34 μM and 11 μM, respectively. The effect of carbachol was inhibited with atropine. The histamine effect was inhibited by H_1-receptor antagonists, but not by H_2- or H_3-receptor antagonists. Arachnoidal AC activity was stimulated by forskolin and prostaglandin D_2 (PGD_2). Histamine did not stimulate AC activity, indicating that H_2-receptors were not present on the cells. In fact, histamine *inhibited* the stimulation of arachnoidal AC by forskolin and PGD_2. In conclusion, porcine arachnoid cells in culture express muscarinic and histamine H_1-receptors linked to PLC, and PGD_2-receptors linked to AC. These receptors may be involved in regulating arachnoid cell dependent permeability of the CSF-blood barrier. Supported by a Burroughs Welcome Osteopathic Fellowship and a grant from the National Multiple Sclerosis Society.

729.6

CRANIECTOMY ACTIVATES DURAL MAST CELLS AND INCREASES CEREBRAL CORTICAL HISTAMINE. Edward L. Orr* and Martha E. Stokely. Department of Anatomy and Cell Biology, U.N.T. Health Science Center, Fort Worth, TX 76107

The dura mater contains numerous mast cells which are degranulated in response to various types of head trauma such as cryogenic lesions (Orr, 1988, Neurochem. Pathol. 8:43-51) or a simple craniectomy (Olesen 1987, Acta Physiol. Scand. 130:63-68. Since degranulated mast cells release large quantities of histamine (HA) and, since exogenous HA can alter the diameter and permeability of pial blood vessels (Yong, et al., 1994, J. Neurotrauma. 11:161-171), the possibility exists that histamine from dural mast cells may cross the meninges to enter the subarachnoid space and affect adjacent brain tissue and pial blood vessels. To test this possibility, adult female Lewis rats were anesthetized by inhalation of methoxyflurane, subjected to unilateral craniectomies, then killed 10 min. later. Samples of cerebral cortex and meninges subjacent and contralateral to the craniectomies were assayed for HA using a specific radioenzymatic assay. Compared to contralateral tissues, the meningeal HA concentration was 62.7 ± 11.8% (mean ± SEM, n=5) of control, while the subjacent cerebral cortical HA concentration was 452.5 ± 165.3% (mean ± SEM, n=5) of control. Since the concentration of HA in the meninges (2.13 ng/mg wet weight) is 140-fold higher than the concentration in the cerebral cortex (0.015 ng/mg wet weight), the decrease in dural HA can easily account for the increased cortical HA observed on the lesioned side of the head. These results suggest that HA, and possibly other products of dural mast cells can cross the meningeal barrier to enter the subarachnoid space and affect underlying brain and associated tissues. (Supported by a grant from the National Multiple Sclerosis Society.)

729.7

STIMULATORY EFFECT OF HISTAMINE ON CALCIUM EFFLUX FROM CULTURED BOVINE ADRENAL CHROMAFFIN CELLS. H. Houchi *, K. Kitamura, K. Minakuchi, Y. Ishimura, M. Okuno, T. Ohuchi and M. Oka. Department of Pharmacology, Tokushima University School of Medicine, Kuramoto, Tokushima, 770, Japan.

Histamine is known to have several effects on adrenal chromaffin cells mediated by its H_1 and H_2 receptors. The histamine H_1 receptor is associated with secretion of catecholamine, accumulation of inositol phosphates, increase in the intracellular level of free Ca^{2+} ($[Ca^{2+}]_i$), synthesis of opioid peptides and phosphorylase of tyrosine hydroxylase in bovine adrenal chromaffin cells. On the other hand, the histamine H_2 receptor is associated with accumulation of cyclic AMP in these cells.

In this study, the effect of stimulation of the histamine receptor on Ca^{2+} mobilization in cultured bovine adrenal chromaffin cells was examined. Histamine (10^{-5}M) increased $[Ca^{2+}]_i$ to a peak in the presence or absence of extracellular Ca^{2+}, followed by decrease with time. Histamine (10^{-8} - 10^{-5}M) also stimulated $^{45}Ca^{2+}$ efflux from cultured bovine adrenal chromaffin cells in a concentration dependent manner. Its stimulatory effect on $^{45}Ca^{2+}$ efflux was inhibited by the specific histamine H_1 receptor antagonist mepyramine. The increase in histamine-stimulated $^{45}Ca^{2+}$ efflux was inhibited by deprivation of extracellular Na^+ and by the Na^+/Ca^{2+} exchange inhibitor amiloride. In addition, histamine stimulated $^{22}Na^+$ influx into the cells, and this action was inhibited by amiloride.

These results suggest that stimulation of the histamine H_1 receptor induces extracellular Na^+-dependent Ca^{2+} efflux from cultured bovine adrenal chromaffin cells, probably acceleration of Na^+/Ca^{2+} exchange.

729.8

DETERMINATION OF AGONIST OR ANTAGONIST ACTIVITY AT THE MELATONIN RECEPTOR BY AN AFFINITY SHIFT METHOD. A. M. Lovelace, R. F. Bruns*. Eli Lilly and Company, Indianapolis, IN 46285.

Receptors exist in resting and activated conformations. Agonists bind preferentially to the activated state. In the present study, we investigated whether this phenomenon can be used to distinguish agonists from antagonists at the melatonin receptor. Binding of [125I]2-iodomelatonin to chicken brain membranes was measured in the presence of 10 mM $MgCl_2$ (activated state) and 1mM EDTA, 100 μM GTP, and 150 mM NaCl (resting state). The Ki of melatonin under activated conditions was 0.22 nM and under resting conditions was 1.70 nM, a 7.04 fold shift in affinity. In contrast, the antagonist luzindole had an activated state Ki of 0.79 nM and a resting state Ki of 0.80 nM, a shift of 1.01 fold. This confirms that the affinity shift method can distinguish melatonin receptor agonists from antagonists.

730.1

THE SYNAPTIC VESICLE GLUTAMATE UPTAKE SYSTEM: RECONSTITUTION WITH ATPASE AND A PARTIALLY PURIFIED GLUTAMATE TRANSLOCATOR. S. M. Lewis*† and T. Ueda†‡. Mental Health Research Institute†, the Departments of Pharmacology and Psychiatry‡, Medical School, University of Michigan, Ann Arbor, MI 48109.

The synaptic vesicle glutamate uptake system has been proposed to play an important role in directing glutamate to the neurotransmitter pathway, away from the metabolic pathway, and has been extensively characterized. Glutamate uptake by synaptic vesicles requires both proton-pump ATPase and glutamate transport activity. The goal of this study is the separation of these two functional components. A fraction rich in ATPase activity, but poor in ATP-dependent glutamate uptake, was obtained by subjecting synaptic vesicles to CHAPS extraction. A fraction with minimal ATPase activity, exhibiting little or no ATP-dependent glutamate uptake activity, was prepared by extracting synaptic vesicles with cholate, followed by glycerol density gradient centrifugation (this fraction was distinct from the major ATPase peak). When these two solubilized fractions were combined into proteoliposomes, significant ATP-dependent glutamate uptake was observed. This suggests that the second fraction contains a partially purified glutamate transporter, largely free of ATPase. This represents the first evidence for the physical separation of the puntative glutamate translocator from the ATPase. This work was supported by NIH grants NS 26884, 1 F32 NS09737-01, and 5-T32-MH15794.

730.2

SH-GROUPS OF CYSTEINE RESIDUES ON GLUTAMATE TRANSPORTERS ARE CRITICAL FOR UPTAKE ACTIVITY. D.Trotti, N.C.Danbolt*, D.Rossi, G.Racagni, O.Gjesdal*, J. Storm-Mathisen** and A.Volterra. Ctr. of Neuropharmacol., Inst. Pharm. Sci., Univ. of Milano, Italy 20133 and *Dept. Anatomy, Univ. of Oslo, Norway P.O.Box 1105.

L-glutamate is the major excitatory transmitter in brain and its transport represents the mechanism by which it is removed from the synaptic cleft and kept below toxic levels. So far, three different glutamate transporters (glu-tps) from rat brain have been cloned: GLT1, GLAST (glial origin) and EAAC1 (neuronal). In this study, we address the possibility that SH-groups of cysteine residues on glu-tps are critical and regulate their uptake activity. We have tested the effect of $HgCl_2$ 0.1-10 μM, a specific SH reagent, on transport activity by (1) a purified to homogeneity rat brain glu-tp (found to be GLT1) and (2) EAAC1, GLAST, GLT1 clones expressed in HeLa cells. Purified or recombinant proteins were functionally reconstituted in artificial liposomes as described (Biochemistry 29:6734-40,1990; Nature 360:464-7,1992). In the purified preparation, $HgCl_2$ decreased transport activity within 1 min in a dose dependent manner with ~40% of inhibition at 10 μM. The inhibition remained following gel-filtration which removes not reacted Hg^{++}. However transport activity could be fully restored by addition of DTT 3mM, a specific disulphide reducing agent. Kinetic uptake parameters were evaluated after exposure to $HgCl_2$ and gel-filtration. Km was not affected, while Vmax was reduced suggesting a non-competitive mode of inhibition. Hg^{++} was found to inhibit also all three the recombinant transporter subtypes, with highest efficacy against GLT1. Parachloromercuribenzoic acid (pCMB 1-100 μM), another SH-reagent also dose dependently inhibit uptake activity mediated by the purified and reconstituted glu-tp. Our data indicate that a modification of SH groups on cysteine residues of glu-tps strongly affects uptake function.

This work was partly supported by Telethon-Italy grant # 586

730.3

MOLECULAR CHARACTERIZATION AND DEVELOPMENTAL EXPRESSION OF A MURINE HIGH-AFFINITY GLUTAMATE TRANSPORTER. M.L. Sutherland[a,b]*, T.A. Delaney[b], J.L. Noebels[a,b]. [a]Division of Neuroscience and [b]Department of Neurology, Developmental Neurogenetics Laboratory, Baylor College of Medicine, Houston TX 77030.

Glutamate is the major excitatory neurotransmitter of the mammalian central nervous system (CNS) acting as a ligand for glutamate metabotropic, AMPA/KA and NMDA receptor subtypes. Transport through high-affinity, Na^+-dependent uptake proteins located in the plasma membrane of presynaptic terminals and surrounding glial cells is the mechanism that maintains glutamate below neurotoxic levels in the synaptic cleft. Through Rt-PCR we have cloned the mouse glutamate transporter (mEAAT2) and demonstrated CNS-specific transcript expression though PCR and southern blot analysis. High-affinity transport of D-aspartate, K_m value (17 $\mu M \pm 5$) was determined in a vaccinia/T7 RNA polymerase expression system. The predicted amino acid sequence of mEAAT2 shares 93 and 96% identity with the human EAAT2 and rat GLT1 homologues, respectively. RFLP analysis and C57BL/6J X *M. Spretus* backcross DNA hybrid panel screening localizes the mEAAT2 gene to mouse chromosome 2, in the vicinity of the recessive neurological mouse mutant, *anx*.

In situ hybridization analysis indicates that the expression of the mEAAT2 transcript during embyrogenesis is CNS-specific and localized to the E15, E17 and E19 day telencephalon, hindbrain and spinal cord. During postnatal development the mEAAT2 transcript is expressed in astrocytes throughout the neuroaxis and in neurons of the cortical subplate and pyramidal cell layer of the hippocampus. At the single cell level, the signal for mEAAT2 mRNA is found not only over cell bodies but also in dendritic, Bergman glia and astrocytic processes. The mEAAT2 mRNA *in situ* distribution provides a clear demonstration that this transporter has both a neuronal and glial localization.

730.4

MODULATION OF GLUTAMATE TRANSPORTER PROTEINS IN AMYGDALA-KINDLED ANIMALS. H.K. Prince Miller*, J.D. Rothstein, P.J. Conn and A.I. Levey. Departments of Neurology and Pharmacology, Emory University, Atlanta, GA 30322 and Department of Neurology, The Johns Hopkins University, Baltimore, MD 21205.

There has been increasing evidence for elevated levels of glutamate in brain regions thought to play a role in the induction and propagation of seizures. Theoretically, this phenomenon could be mediated by modulation of high-affinity uptake systems for glutamate. To date, three distinct glutamate transporters have been cloned: GLT-1, GLAST and EAAC-1. Immunocytochemistry studies have shown that GLT-1 and GLAST are localized to glia, whereas EAAC-1 is localized to neurons. We investigated whether there were changes in the levels of these transporters in rats that had undergone kindling-induced epilepsy by stimulation of the amygdala. Levels of GLT-1, GLAST and EAAC-1 were examined in three brain regions (hippocampus, piriform cortex/amygdala and limbic forebrain) by quantitative immunoblotting using subtype specific antibodies. Immunoblotting revealed a 60% decrease in GLAST protein in the piriform cortex/amygdala region 24 hours after the last seizure. No changes in GLAST were observed in the other regions that were examined. EAAC-1 levels were found to be increased to 130% of control in the hippocampus, with no changes in the other regions examined. No changes in GLT-1 were observed in any region examined. Future studies will be aimed at determining whether these changes result in any functional consequence to the levels of extracellular glutamate. *HKPM is the recipient of a PhRMA F predoctoral award.*

730.5

POSTSYNAPTIC UPTAKE OF EXCITATORY AMINO ACIDS IN RAT CEREBELLAR PURKINJE CELLS. [1]Y. Kataoka, [1]H. Ohmori and [2]K. Kataoka*. [1]Dept. of Physiology, Kyoto Univ., Kyoto, Kyoto 606-01 and [2]Dept. of Physiology, Ehime Univ. Sch. of Med., Shigenobu, Ehime 791-02.

Postsynaptic activities of released excitatory amino acids (EAAs) are known to be terminated by uptake mechanisms into presynaptic neurons and glial cells. The existence of postsynaptic uptake system of EAAs has not been confirmed. We found a current component which was induced by EAAs (L-glutamate, L-aspartate and L-homocysteate) and was not suppressed by the antagonist for NMDA receptors (200 μM APV) and by the antagonist for non-NMDA receptors (20 μM CNQX) in rat cerebellar Purkinje cells with the whole cell patch recording technique. We call this current as the APV & CNQX resistant current. Dose dependencies of three amino acids for inducing this current had dissociation constants from 15.7 μM to 25.0 μM. The induction of this current did not increase current noise, and was suppressed by a removal of Na^+ from extracellular medium or by an application of the inhibitor for high affinity glutamate transporters (D-threo-β-hydroxyaspartate). The APV & CNQX resistant current was induced all over the subcellular regions studied by ionophoretic application of EAAs including dendrites and cell somata. These observations indicate that the APV & CNQX resistant current is likely induced by the Na^+ dependent high affinity uptake mechanism of EAAs in postsynaptic Purkinje cells.

730.6

GLUTAMATE UPTAKE INHIBITORS: DIFFERENTIATION OF SUBSTRATES AND NON-SUBSTRATES. H. Koch*, F. Lovering+, S. Esslinger+, A.R. Chamberlin+, and R.J. Bridges. Dept of Pharm. Sci., Univ. of Montana, Missoula MT 59812, Dept. of Chem.+, Univ. of Calif., Irvine CA 92717.

High-affinity, sodium-dependent glutamate transport plays a key role in the regulation of extracellular glutamate and the prevention of excitotoxic damage. During the course of delineating transporter pharmacology, we identified several conformationally constrained analogues of glutamate that potently block the uptake of ^{3}H-D-asparatate into rat cortical synaptosomes, *e.g.*, L-*trans*-2,4-pyrrolidine dicarboxylate (L-*trans*-2,4-PDC), L-*anti-endo*-3,4-methano-pyrrolidine dicarboxylate (L-*anti-endo*-3,4-MPDC) and *meso*-3,4-MPDC. A key question that remains to be answered is whether or not these novel inhibitors are also substrates that can be translocated into the synaptosomes. While all competitive blockers would be expected to bind to the transporter, it is likely that inhibitors can be further subdivided between substrates and non-substrates. In lieu of preparing radiolabeled derivatives, we have addressed this issue by quantifiyng the ability of the analogues to participate in the process of heteroexchange. Compounds that act as substrates stimulate the rate of exodus of ^{3}H-D-asparatate previously loaded into the synaptosomes. Using this approach we find that while each of the three structurally constrained PDC analogues exhibited similar abilities to block uptake (Ki values 1-6 μM), the compounds varied significantly in their ability to serve as substrates. The observed rank order of exchange with ^{3}H-D-asparatate was *meso*-3,4-MPDC>L-*trans*-2,4-PDC>L-*anti-endo*-3,4-MPDC, with rates comparable to about 90, 75, and 40% of that produced by L-glutamate, respectively. Knowledge of an uptake inhibitor's ability to serve as a substrate is a key factor in interpreting its actions in physiological investigations. Modeling studies have been initiated to delineate the chemical basis that differentiates transportable and non-transportable inhibitors. This work was supported in part by NIH/NINDS NS 30570 and NS 27600.

730.7

INHIBITION OF HIGH AFFINITY GLUTAMATE UPTAKE BY AMINO ACID CARBAMATES. R.J. Bridges*, C.A. Baker, and P.B. Nunn+. Dept. of Pharm. Sci., Univ. Montana, Missoula, MT, 59812 and Biomed. Sci. Div. +, King's College London, England.

A number of amino acids have been identified that are capable of interacting with CO_2 and forming carbamates that induce excitotoxic neuronal injury, *e.g.*, β-N-methylamino-L-alanine (BMAA) and α,β-diaminopropionic acid (DAP). Although the parent amino acids bear little resemblance to glutamate, the resulting carbamate can closely resemble the dicarboxylic amino acid character shared by most EAA agonists. In the present study a number of these potentially neurotoxic amino acids were tested to determine if their resulting carbamates could also inhibit high-affinity, sodium-dependent glutamate transport. Amino acids were assayed, in the presence and absence of $NaHCO_3$ (20 mM), as blockers of the uptake of ^{3}H-D-aspartate into rat forebrain synaptosomes. Compounds found to be inactive as inhibitors, either by themselves or as carbamates, included D- and L-cysteine, D- and L-serine, D- and L-α,γ-diaminobutyric acid (DAB), and BMAA. In contrast, we found that L-DAP was a potent inhibitor when added with $NaHCO_3$, and that the extent of the inhibition was significantly reduced in the absence of $NaHCO_3$. This activity was stereoselective, as L-DAP was considerably more potent as an inhibitor than D-DAP. Kinetic plots yielded patterns consistent with a competitive inhibitor (Ki ≈ 5 μM). Further, preliminary studies demonstrated that the L-DAP carbamate could undergo heteroexchange with ^{3}H-D-aspartate preloaded into synaptosomes, suggesting that it was not only an inhibitor, but also a substrate. While the CO_2 may interact with either amino group on L-DAP, molecular modeling studies indicated that the β-carbamate is the likely active inhibitor. These results suggest that the neurotoxic action of DAP, but not BMAA, may include the action of its carbamate not only at EAA receptors, but also at the high-affinity glutamate transporters. This work was supported in part by NIH/NINDS NS30570.

730.8

CALCIUM-INDEPENDENT EXTRACELLULAR ALKALINE SHIFTS EVOKED BY GLUTAMATE. S.E. Smith* and M. Chesler Depts.Physiol./Neurosurg. NYU Med. Ctr., 550 1st Ave., NY, NY 10016.

Extracellular alkaline shifts evoked by glutamate (GLU) receptors are generated by a Ca^{2+}-dependent mechanism [1, 2]. We tested whether other alkalinizing processes are unveiled with zero Ca^{2+} (EGTA) media. Alkaline shifts were evoked in hippocampal slices (CA1) by pressure ejection of agonists near a pH microelectrode. In zero Ca^{2+}, GLU, AMPA and NMDA caused no alkaline shift, or a small acidification. Slowing buffer kinetics with the carbonic anhydrase inhibitor benzolamide (10 μM) uncovered a slow alkalinization evoked by GLU (0.03 ± .01 pH units, n=5 slices) but not by AMPA, NMDA or the metabotropic agonist trans-ACPD. A similar Ca^{2+}-independent alkaline shift was noted following ejection of L-trans-2,4-PDC (0.05 ± .01 pH units n=6), a GLU uptake inhibitor transported by the GLU carrier. Both GLU and L-trans-2,4-PDC-evoked alkaline shifts persisted in the presence of APV (50 μM) and CNQX (≤ 100 μM). These observations are consistent with efflux of base by the GLU transporter [3]. Since detection of the Ca^{2+}-independent mechanism required greatly diminished buffering, its contribution to bulk extracellular alkalinization is normally minimal. Supported by NIH grant NS32123. [1] Paalasmaa et al. (1994) *J. Neurophys.* 72(4):2031. [2] Smith et al. (1994) *NeuroReport* 5:2441. [3] Bouvier et al. (1992) *Nature* 360:471.

730.9

EXPRESSION OF NEURONAL AND GLIAL GLUTAMATE TRANSPORTERS IN THE RAT OPTIC NERVE. I. Choi, S. Y. Chiu* and J. D. Rothstein. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706; Johns Hopkins Univ., Dept. of Neurology, Baltimore, MD 21287.

The rat optic nerve consists only of axons and glia, and are devoid of classical synapses. Recent studies, however, revealed dynamic axon-glial signaling in this nerve postulated to be mediated by non-vesicular release of glutamate (Kriegler & Chiu, 1993). One release mechanism for glutamate in the optic nerve is reversal of high-affinity glutamate transporters. Here we examined the expression of two glial glutamate transporters (GLAST and GLT) and one neuronal transporter (EAAC1) in the rat optic nerve. RT-PCR analysis revealed the presence of mRNAs for GLT and GLAST, but not EAAC1, in rat optic nerves. RNase protection assays were used to quantify the mRNA expression using a housekeeping gene, GAPDH, to control for RNA loading. Of the two glial transporters, mRNA for GLAST is expressed about 20 times higher than GLT, both in developing and in adult rat optic nerves. GLAST mRNA level in the optic nerve is about 2-fold higher than in cortex (grey matter). Intriguingly, optic nerve expresses slightly higher level of GLAST mRNA than cerebellum, a brain region previously shown to express the the highest level of GLAST. Developmentally, GLAST mRNA level in the rat optic nerve is highest at P2, and drops slightly by adult. Nerve transection results in little or no changes in mRNA levels for GLAST and GLT at 4-6 days post-transection. Western blot analysis performed on rat optic using peptide specific antibodies raised against GLT, GLAST and EAAC1 reveals major bands of 72, 70, and 70 kDa respectively. Light-level immunocytochemistry shows that GLT and GLAST stain glial processes, EAAC1 stains axons. In conclusion, we suggest that these glial and neuronal transporters mediate neurotransmitter signaling in the optic nerve, by modulating glutamate level in the periaxonal space during nerve activity. In particular, the unusually high level of expression of GLAST in the optic nerve suggests novel roles for this glial transporter in the optic nerve.

730.10

EFFECTS OF NATRIURETIC PEPTIDES ON SODIUM TRANSPORT AND GLUTAMATE UPTAKE IN RAT BRAIN ASTROCYTES. C.F. Deschepper*, S. Picard, K.L. Grove, E.L. Schiffrin and R. Touyz, IRCM, Montréal, Canada H2W 1R7

Natriuretic peptides (NP) in the brain have been shown to control a variety of neuronal functions. The actions of these peptides are generally believed to be mediated via the intracellular generation of cGMP. Since astrocytes appear to be the only brain cells generating cGMP in response to NP, we investigated the effects of C-type NP (CNP) and/or cGMP on secondary cultures of rat brain type-1 astrocytes. We observed that CNP 10^{-6} M diminished intracellular free sodium (as determined by fluorescence of SBFI) in astrocytes. Conversely, intracellular pH (as determined by fluorescence of BCECF) was decreased in a dose-dependent fashion by exposure to CNP. Both effects were mimicked by membrane-permeant analogs of cGMP, and were most likely to be carried out by the Na^+-H^+ antiporter, since they were blocked by pre-incubation of the cells with the specific inhibitor 5-N,N-(hexamethylene)-amiloride (HMA). In contrast, CNP and/or cGMP analogs had no effects on Na^+-K^+-ATPase function nor on Na^+-K^+-$2Cl^-$ transport. We also found that both CNP 10^{-6} M and 8-bromo cGMP 10^{-3} M diminished the uptake of [^{3}H]-glutamate by lowering the V_{max} but without affecting the K_m of this transport. This effect could be explained partially by inhibition of the Na^+-H^+ antiporter, since HMA had a similar effect. However, glutamate uptake could be decreased even further by CNP in HMA-pretreated cells, indicating that this peptide could affect glutamate uptake by an additional mechanism independent of Na^+-H^+ exchange. Activation of protein kinase G (PKG) is the most likely mechanism, since the effect of CNP was blocked by KT5823, a specific PKG inhibitor. The effects of NP on Na^+-H^+ exchange and glutamate uptake in astrocytes may constitute mechanisms by which these cells could enhance the actions of glutamate on neighboring neurons. (Supported by MRC and HSF Canada).

730.11

EPSC SHAPING, AND GLUTAMATE RELEASE IN ISCHAEMIA, BY GLUTAMATE UPTAKE CARRIERS Michiko Takahashi, Brian Billups, Monique Sarantis and David Attwell*. Dept. of Physiology, University College London, Gower St., London, WC1E 6BT, England.

Glutamate uptake is often thought of as being presynaptic or in glial cells, but immunostaining suggests that rat cerebellar Purkinje cells express EAAC1 carriers (Neuron 13, 713). By applying glutamate analogues iontophoretically to Purkinje cells in cerebellar slices, we detected a membrane current component attributable to glutamate uptake. To investigate the role of this postsynaptic uptake, currents evoked by climbing fibre stimulation were recorded. By altering intracellular ions via the patch pipette, we examined shaping of the EPSC by glutamate uptake. Inhibiting uptake by removing pipette Cs^+ (countertransported by the uptake carrier) or adding D-aspartate to the pipette slowed the EPSC decay. These data suggest that removal of glutamate from the synaptic cleft by postsynaptic glutamate uptake can shape the EPSC.

In brain ischaemia $[K^+]_o$ rises, making the uptake carrier reverse. We monitored reversed uptake from salamander retinal glia electrically, and by detecting Glu^- release from the opening of AMPA receptor channels in rat Purkinje cell bodies positioned near the glia. Acidifying the external solution, as also occurs in ischaemia, decreased both the reversed uptake current, and the release of glutamate produced by glial depolarisation in raised $[K^+]_o$. These data suggest that the acidification occurring in ischaemia could slow the release of glutamate occurring by reversed uptake, and thus protect neurons against transient (but not sustained) ischaemia.

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730.12

COMPARISON OF GLUTAMATE TRANSPORTER GLT-1 mRNAs IN BRAIN AND PERIPHERAL TISSUES. Y. Kanai*, N. Tate, H. Endou. Dept. of Pharmacology and Toxicology, Kyorin Univ. Sch. of Med., 6-20-2 Shinkawa, Mitaka, Tokyo 181, Japan.

Glutamate transporters play central roles to keep extracellular glutamate concentration below neurotoxic level in the central nervous system. In peripheral tissues, glutamate transporters are also important to absorb glutamate from intestine and kidney epithelia and to provide cells with glutamate for nutritional purpose. In this study, we show that glutamate transporter GLT-1 which is expressed in astrocytes in brain is also expressed in peripheral tissues such as liver and testis in mice. In contrast to 11.3kb mRNA in brain, liver and testis express 2.2 kb mRNA. We have isolated four GLT-1 cDNAs from random primed mouse brain cDNA library and five GLT-1 cDNAs from oligo dT primed mouse liver cDNA library. Mouse brain GLT-1 shows 98% amino acid sequence identity to rat brain GLT-1 and has the identical N-terminus. Liver type GLT-1, however, exhibits difference in its predicted N-terminus amino acid sequence compared to brain GLT-1. When expressed in *Xenopus laevis* oocytes, both brain and liver GLT-1s induce Na^+-dependent L-glutamate uptake. Liver GLT-1 mRNA uses polyadenylation sites close to the stop codon for polyA tail addition. Brain and liver GLT-1 cDNAs have different 5'-untranslated regions. This suggests that GLT-1 mRNA is alternatively spliced at the 5'-ends and that brain and peripheral tissues possess different mechanisms for the regulation of expression of GLT-1 gene.

730.13

DIFFERENTIAL EXPRESSION OF THE GLUTAMATE TRANSPORTER SUBTYPES DURING MOUSE BRAIN DEVELOPMENT. Takashi Shibata, Masahiko Watanabe, Kohichi Tanaka and Yoshiro Inoue*. Dept. of Anatomy, Hokkaido Univ. Sch. of Med., Sapporo 060, and Dept. of Degenerative Neurological Diseases, National Inst. of Neuroscience, NCNP, Tokyo 187, Japan.

The glutamate transporter terminates physiological action of excitatory amino acid glutamate in the synapse. In the present study, *in situ* hybridization was employed to clarify developmental regulation of three glutamate transporter subtypes (GluT1 (GLAST), GLT1, and EAAC1) in the mouse brain from embryonic day 13 to postnatal day 120. The GluT1 mRNA was prominently expressed in the ventricular (proliferative) zone throughout the brain during embryonic stages. In the late embryonic and early postnatal development, the expression in the ventricular zone gradually diminished and disappeared, while that in the mantle zone increased progressively. In particular, the expression increase was outstanding in the superficial layer of the cerebellum (Bergmann glial cells). The GLT1 mRNA was also expressed in the ventricular zone, but its expression was virtually restricted to the telencephalon at perinatal stages. During the second postnatal week, the expression levels were augmented prominently in the telencephalon, including the cerebral cortex, hippocampal CA3, and septum. In contrast, the EAAC1 mRNA in the ventricular zone was below the detection threshold at any developmental stages. According to postnatal maturation, signals for the EAAC1 mRNA increased gradually over the brain gray matter, with higher levels in the hippocampal CA1 region and dentate gyrus. These findings suggest that the glutamate transporter may play an important role in early differentiation of the brain, in which the transporter subtypes would be involved differentially.

730.14

GLIAL SWELLING BY AP3 AND GLUTAMATE TRANSPORT INHIBITORS IN THE RETINA C.O. Kirby-Sharkey, Y. Izumi*, S. Mennerick, Ann M. Benz, J. Labruyere, C.F. Zorumski, M.T.Price & J.W.Olney
Dept. of Psychiatry, Washington Univ., School of Med., St.Louis, MO 63110.

Using rat retinal preparations, we observed a characteristic glial swelling induced by amino-3-phosponopropionate (AP3). Although AP3 has been widely used as an inhibitor of metabotropic glutamate receptors (mGluRs), AP3 is also known to have properties of mGluR agonists. Thus, we examined whether the effects of AP3 were through the modulation of mGluRs. However, alpha-methyl-4-carboxyphenylglycine (MCPG), a selective competitive antagonist of mGluRs, 1S3R-ACPD, DHPG or L-CCG-I, agonists of mGluRs, did not produce glial swelling. Moreover, the effects of AP3 were not prevented by the administration of MCPG or 8-bromo-cAMP. These results suggest that the modulation of mGluR, activation or inhibition, was not likely responsible for the glial swelling. Furthermore, the glial swelling by AP3 was not prevented by DNQX and MK-801, antagonists of non-NMDA and NMDA ionotropic receptors.

The glial swelling by AP3 was attenuated by replacement of extracellular Na^{++} but not Cl^-, and was mimicked by D,L-threo-3-hydroxyaspartate (THA), L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC), glutamate transport inhibitors. Moreover, AP3 produced a Na^{++} inward current in hippocampal glial cells that was occluded by THA. Like THA, AP3 also prolonged glutamate-mediated synaptic currents in hippocampal neurons suggesting that AP3 inhibits glutamate uptake. These results suggest that AP3 induces glial swelling in the retina by serving as a substrate for glutamate transporters. Taken together, AP3 may be a useful substrate to elucidate the mechanism of glial swelling, which is also produced by glutamate but is not prevented by DNQX, MK-801 or MCPG.

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730.15

FUNCTIONAL CHARACTERIZATION AND LOCALIZATION OF A HUMAN EXCITATORY AMINO ACID TRANSPORTER WITH PROPERTIES OF A LIGAND-GATED CHLORIDE CHANNEL. W.A. Fairman, R.J. Vandenberg, J.L. Arriza, E.M. Shannon, G.H. Murdoch*, M.P. Kavanaugh, & S.G. Amara Howard Hughes Medical Institute and Vollum Institute, Oregon Health Sciences University, Portland, Oregon 97201.

The reuptake of excitatory amino acids (EAA's) plays an important role in regulating synaptic and extracellular concentrations of glutamate. The functional properties of a novel member of the glutamate transporter gene family, EAAT4, were examined in *Xenopus* oocytes. Application of L-aspartate or L-glutamate to EAAT4 expressing oocytes generated an inward current at hyperpolarized potentials and an outward current at depolarized potentials. In contrast to other members of this gene family, the EAA-induced current in EAAT4 expressing oocytes had a linear voltage dependence and reversed near the chloride equilibrium potential. This EAA-elicited current is not solely due to the movement of cotransported ions, but also appears to reflect a ligand-activated chloride conductance associated with the carrier. The reversal potential of the L-aspartate-induced chloride conductance shifted 57 ± 1.5 mV per ten fold change in $[Cl^-]_o$, approaching the Nernst prediction for a chloride selective conductance. Both the EAA-induced chloride conductance and the radiolabeled amino acid flux are sodium-dependent, but flux does not appear to depend on extracellular chloride. Comparison of the current and radiolabeled flux observed under voltage clamp conditions suggest that the chloride conductance represents greater than 95% of the L-aspartate induced current at -60mV. Additional studies demonstrate that EAAT4 does not appear to activate any known endogenous chloride channels in oocytes. Northern blotting analysis indicates that EAAT4 is most abundant in the cerebellum, with *in situ* hybridization studies indicating that this carrier prominently expressed in Purkinje cells. The chloride conductance associated with EAAT4 is activated by micromolar concentrations of L-glutamate and L-aspartate at physiologically relevant membrane potentials. Thus, EAAT4's dual properties as a ligand-gated chloride channel as well as a transporter could regulate neuronal excitability and serve as a monitor of extracellular EAA levels.

730.16

TOPOLOGICAL STUDIES OF THE SODIUM-DEPENDENT GLUTAMATE TRANSPORTER FAMILY [1,2]R.P. Seal, [2]J.L. Arriza and [2,3]S.G. Amara*
[1]Program in Neuroscience, [2]Vollum Institute and [3]Howard Hughes Medical Institute, Oregon Health Science Univ., Portland OR. 97201

In contrast to several other transporter families which appear to contain twelve α-helical transmembrane (TM) segments, the precise number and orientation of TM domains in the sodium-dependent glutamate carriers remains unclear. Hydropathy analyses of four human glutamate transporters, EAATs1-4, suggests the presence of six to ten α-helical TM domains. To resolve this apparent ambiguity, the membrane topology of the glutamate carriers was investigated by multiple strategies. A FLAG epitope was introduced into the EAAT1 subtype in several domains. The membrane orientation of the epitope for each FLAG-tagged construct was determined by transfecting mammalian cells and carrying out immunofluorescence under permeabilized and non-permeabilized conditions, as well as by assessing protease sensitivity of the epitope in a microsomal membrane-coupled translation system. To examine whether putative TM segments span the membrane, a series of C-terminal truncations of EAAT1 were made following putative TM segments and each deletion was linked to a prolactin reporter epitope. These constructs were expressed in the *in vitro* system and examined for protease sensitivity of the prolactin reporter as a determinant of membrane orientation. The results of these experiments support a model in which both the N- and C-termini are intracellular, the loop between TM domains three and four is extracellular and the first six hydrophobic segments span the membrane, most likely as α-helices. Studies of the last four putative TM segments (7-10) suggests that they are membrane associated, but may not span the membrane as α-helices. Further investigation into the topology of this region is currently underway.

730.17

Isolation and Characterization of Mouse Excitatory Amino Acid Transporter(EAAT) Genes Marc A. Kirschner[1,2], and Susan G. Amara[2,3], Oregon Health Sciences University, Portland, Oregon 97201. Department of Neurology[1], the Vollum Institute for Advanced Biomedical Research[2] and the Howard Hughes Medical Institute[3].

A family of four distinct high-affinity sodium-dependent excitatory amino acid transporter cDNAs has been isolated from mouse. These reuptake carriers may modulate neurotransmitter concentrations at the excitatory glutamatergic synapses of the mammalian central nervous system. Mouse EAAT subtypes were isolated from a λzap cDNA library with PCR-generated probes. PCR reactions were performed on mouse cerebellum cDNA with degenerate oligonucleotide primers homologous to highly conserved regions of human and rat EAAT cDNAs. The mouse EAATs show similarity to subtypes identified in other species. Functional expression of specific subtypes demonstrates apparent affinity constants(K_t's) in the 40 to 60 μM range--values comparable to those obtained for corresponding subtypes isolated from other species. Each transporter is prominently expressed in the CNS. EAAT2 and EAAT4 transcripts are principally found in the CNS. However, EAAT3 is highly expressed in kidney whereas EAAT1 is expressed in lung and skeletal muscle outside the CNS.

EAAT genes have been mapped in mouse and human genomes and subtypes have been found to lie on syntenic chromosome regions. Neuroexcitability loci that may have a role in mouse models of epilepsy have been found near *Eaat2*. *Eaat2* is also located in the vicinity of two mouse recessive disorder loci, fidget(*fi*) and anorexia (*anx*).

We have also isolated and characterized 4 distinct EAAT genomic clones from a λDASH library using full length cDNAs as probes. These range in size from 14 to 18 kb. Further study of EAATs will elucidate their role in mammalian development and may provide models of neurological disease.

730.18

DIHYDROKAINATE (DHK) BLOCKS L-*TRANS*-PYRROLIDINE-2,4-DICARBOXYLIC ACID (PDC) NEUROTOXICITY AND ^{45}CALCIUM INFLUX IN ASTROCYTE-RICH CORTICAL CULTURES. R.C. Blitzblau, X. Gan*, and P.A. Rosenberg. Dept. of Neurol., Children's Hospital & Harvard Medical School, Boston MA 02115.

PDC is a potent inhibitor of high affinity glutamate uptake (Bridges et al. *J.Med.Chem.* 34: 717-725). Transportable inhibitors of high affinity glutamate uptake such as PDC, DL-threo-β-hydroxyaspartate, and 1-aminocyclobutane-*trans*-1,3-dicarboxylic acid have been shown to be neurotoxic by an NMDA-receptor mediated mechanism that seems to involve reversal of glutamate transport (Blitzblau et al. *Soc.Neurosci.Abstr.* 20: 274). In contrast, the non-transportable inhibitor dihydrokainate (DHK), in concentrations up to 1 mM, had no neurotoxicity in cortical cultures. In these experiments we sought to further test the hypothesis that the toxicity of PDC was mediated by an effect on glutamate transport, by testing whether the toxicity of of the transportable, toxic, uptake inhibitor PDC could be blocked by the non-transportable, non-toxic, uptake inhibitor DHK. We found that 1 mM DHK significantly reduced the toxicity of 1 mM PDC. Survival of neurons in the presence of PDC was increased from 29 ± 9 % to 87 ± 5 % ($n = 3$; $p < .05$). NMDA-receptor activation stimulates the influx of ^{45}Ca in cortical cultures. Exposure of cortical cultures to PDC stimulated ^{45}Ca influx, whereas exposure of cortical cultures to DHK had no effect on ^{45}Ca influx. DHK at 1 mM reduced the influx of ^{45}Ca into cortical cultures exposed to 1 mM PDC to control levels ($n = 2$), supporting the results obtained in the neurotoxicity paradigm.

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730.19

MOLECULAR KNOCKOUT OF NEURONAL, BUT NOT GLIAL, GLUTAMATE TRANSPORT PRODUCES EPILEPSY.

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Glutamate transport is believed to be essential for the inactivation of synaptically released glutamate. Three glutamate transporters have been cloned; EAAC1 is specific for neurons, while GLT-1 and GLAST have an astroglial localization. Both EAAC1 and GLT-1 have the highest brain expression in the hippocampus. To understand the relative role of each transporter in glutamate neurotransmission, antisense oligonucleotides (ODN) were used to selectively inhibit the synthesis of individual transporter subtypes. Antisense ODN were administered intraventricularly over a 7 day period by mini-osmotic pumps (1μL/hr; 10nmoles/day). Hippocampal EAAC1, GLT-1, and GLAST protein levels were each decreased by more than 50% after one week of individual chronic antisense treatment, as determined by semi-quantitative immunoblot analysis. Functional glutamate transport was decreased in hippocampus by about 20% after EAAC1 antisense ODN, 50% after GLT-1antisense ODN, and 20% after GLAST ODN. Furthermore, sense ODN and random ODN (containing identical proportions of each nucleotide) for each transporter did not alter transporter protein levels, functional transport, or have behavioral effects. Preliminary microdialysis studies indicated that antisense knockout of glutamate transporter elevated extracellular glutamate levels. Behaviorally, after 3 days treatment, 4 out of 8 EAAC1 antisense treated animals developed hyper-irritability, and stimulus-evoked tonic seizures. By 7 days of treatment, 7 out of 8 EAAC1 antisense treated rats exhibited tonic, and occasionally tonic-clonic seizures. Only 2 out of 11 GLT-1 antisense treated rats developed tonic seizures and none of the 10 GLAST antisense treated rats developed seizures after 7 days of treatment. These studies suggest that dysfunction of *neuronal* glutamate transport, and to a small extent glial glutamate transport, contributes to glutamate induced seizures.

730.20

STRUCTURE AND FUNCTIONAL CHARACTERIZATION OF GLUTAMATE TRANSPORTER cDNAS AND GENES.

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Glutamate is the major excitatory neurotransmitter in the central nervous system. Like many small neuroactive compounds, it is thought that the physiological action of glutamate is terminated by Na^+-dependent high affinity transport proteins that are found in the plasma membrane of both presynaptic nerve terminals and glial cells.

We have isolated mouse cDNAs and genes encoding three subtypes of glutamate transporters(two are of glial, one of neuronal origin). The amino acid sequences of the three transporters are very similar to each other, displaying ~50% identity and ~60% similarity. The pharmacological properties and the tissue distribution of the mouse three glutamate transporters are almost identical to those of glutamate transporters previously isolated from other species.

Genomic organization of the two subtypes of glial glutamate transporters shows closely related exon-intron arrangements(they contain 10 exons interrupted by 9 introns). On the other hand, the gene encoding the neuronal subtype of glutamate transporter is comprised of 12 exons.

The cloning and functional analyses of mouse three subtypes of glutamate transporter cDNAs and genes provide a basis for future research into the structure, function, pharmacology and the in vivo functional roles of glutamate transporters.

730.21

CHARACTERIZATION OF THE RELEASE OF DOPAMINE AND 1-METHYL-4-PHENYLPYRIDINIUM(MPP+) THROUGH THE RAT DOPAMINE TRANSPORTER EXPRESSED IN COS CELLS.

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In addition to the principal role of neurotransmitter transporter in terminating synaptic transmission by reaccumulating released neurotransmitter, revasal of the transport process participates in the neurotransmitter release observed in a certain physiological condition while distinguishable from vesicular release. To explore physiological relevance of the transporter-mediated release in correlation to the action of psychostimulant such as cocaine or neurotoxin, we characterized the release of dopamine(DA) and the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium(MPP^+) through the rat DA transporter expressed in COS cells. COS cells expressing the transporter revealed the ability to release the preloaded [^{3}H] DA and [^{3}H] MPP^+ in different degrees and time-course. Release of [^{3}H] DA was enhanced in the presence of extracellular DA, but not affected by cocaine or GBR-12935. On the other hand, release of [^{3}H] MPP^+ was enhanced by DA and MPP^+, while it was inhibited by cocaine, nomifensine and GBR-12935. These different properties between the transporter-mediated releases of DA and MPP^+ suggest the different mode of translocation / reorientation of the transporter for these substrates. This might be important for the re-distribution of substrates for the transporter, especially for the neurotoxic substance such as MPP^+.

730.22

GLUTAMATE UPTAKE IS UNCOUPLED FROM THE COUNTERTRANSPORT OF HYDROXYL EQUIVALENTS BY VERY LOW CONCENTRATIONS OF $HgCl_2$.

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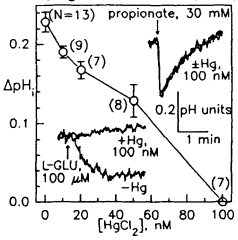
Uptake of L-GLU acidifies astrocyte cytoplasm (Brookes & Turner, *Neurosci. Lett.* 160:73, 1993) consistent with OH^- countertransport (Bouvier et al., *Nature* 360:471, 1992). We examined the effect of $HgCl_2$ on intracellular pH (pH_i) using the fluorescent pH indicator BCECF in mouse cerebral astrocytes grown on glass coverslips. In HEPES/Tris-buffered solution (pH 7.4, 35°C; containing ~0.1 mM HCO_3^- derived from air), 100 nM $HgCl_2$ transiently acidified pH_i by 0.095±0.008 (SE, N=13), but did not affect the response of pH_i to acid loading with 30 mM propionate or to alkaline loading with 10 mM methylamine, indicating that buffering capacity and acid extrusion were unaffected. Alkalinization induced by 9 mM K^+ (Brookes & Turner, *Am. J. Physiol.* 267:C1633, 1994) was 52% inhibited ($p<0.001$) by 100 nM $HgCl_2$, but unaffected by 50 nM. Acidification induced by 100 μM L-GLU (ΔpH_i, see Fig.) was completely blocked by 100 nM $HgCl_2$, and partially blocked by [$HgCl_2$] as low as 10 nM ($p<0.05$). Yet, uptake of 100 μM [^{3}H]-L-GLU was not inhibited by 100 nM $HgCl_2$ (Brookes & Kristt, *J. Neurochem.* 53:1228, 1989; confirmed here). Possible interpretations are that, in the presence of very low [$HgCl_2$], the Na^+-L-GLU symport can countertransport Cl^- or $HgCl_3^-$, or is uncoupled from anion countertransport. (Supported by USPHS grant ES03928).



730.23

DOWN REGULATION OF GLIAL GLUTAMATE TRANSPORTERS FOLLOWING FIMBRIA-FORNIX (FF) TRANSECTIONS AND CORTICOSTRIATAL LESIONS.

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High-affinity sodium-dependent glutamate transport is the primary means to maintain low extracellular glutamate levels. Three glutamate transporters have been cloned: EAAC1 is localized to neurons, while GLT-1 and GLAST are astroglial. The expression of glutamate transporter subtypes in rats was evaluated following: 1) unilateral FF transections to disrupt glutamatergic hippocampal/subicular efferents to the septum and hypothalamus, or 2) unilateral lesions of motor-sensory cortex to remove glutamatergic corticostriatal input. The hippocampus and striatum were collected from animals sacrificed at 3, 7, 14, and 30 days and tissue homogenates were 1) assayed for functional glutamate transport and 2) immunoblotted using GLT-1, GLAST, EAAC1, and GFAP, a marker for astrocytes. GLT-1 and GLAST immunoreactivity was significantly decreased in hippocampus ipsilateral to the FF transections at 7 and 14 days postlesion, and total hippocampal glutamate transport was decreased ipsilaterally by 50% at 7 and 14 days postlesion. GLT-1 and GLAST immunoreactivity was significantly decreased within the striatum ipsilateral to the cortical-callosal lesions at 7 and 14 days postlesion, and total striatal glutamate transport was decreased ipsilaterally by 60% at 7 and 14 days postlesion. By 30 days postlesion, glial glutamate transporter protein immunoreactivity and functional glutamate transport returned to control levels. The decrease in astrocytic glutamate transporters was associated with an increase, rather than a decrease, in GFAP immunoreactivity. No significant changes in EAAC1 immunoreactivity were observed at any of the postlesion time points. This study demonstrates that alterations in glial, rather than neuronal, glutamate transporters occur following axotomy of glutamatergic pathways. We hypothesize that glial glutamate transporters may play a dynamic role in plasticity associated with hippocampal and striatal disconnection.

730.24

LOCALIZATION AND CHARACTERIZATION OF HIGH AFFINITY GLUTAMATE TRANSPORTERS IN RAT RETINA.

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The major component of excitatory neurotransmission in the mammalian central nervous system is mediated by L-glutamate. Termination of such neuronal activity is achieved by sodium-dependent, high affinity uptake systems located in glial cell processes or in presynaptic nerve endings. To date, three related but distinct glutamate transporters have been cloned: GLT-1 and GLAST-1 from rat brain as well as EAAC-1 from rabbit intestine. The retina provides an excellent model system to investigate the cellular localization, function and molecular properties of these transporter molecules, because of its highly ordered anatomical organization, particularly with regard to the glutamatergic circuitry.

Reverse transcribed-polymerase chain reaction of rat retina mRNA, using specific primers for GLAST-1, GLT-1 and EAAC-1, revealed the presence of message for all three transporter subtypes in the retina. Immunocytochemistry indicated that Müller glial cells are exclusively reactive for GLAST-1, whereas GLT-1 is restricted to cone photoreceptors and a subpopulation of cone bipolar cells. Comparative immunoblots of retinal membrane proteins using polyclonal antibodies against GLAST-1 and GLT-1 revealed a significantly higher content of GLAST-1 transporter protein in retinal tissue. Additionally, accumulation of L-[^{3}H]glutamate occurs predominately in Müller glial cells.

These results suggest that Müller glial cells are responsible for the removal of most synaptically released glutamate in the retina, and that this uptake is mediated by GLAST-1.

730.25

MOLECULAR CHARACTERIZATION OF GLUTAMATE TRANSPORTER SUBTYPES IN THE SALAMANDER RETINA. J.L. Arriza*, S.D. Eliasof, M.P. Kavanaugh, C.E. Jahr, and S.G. Amara, Vollum Institute, OHSU, Portland OR 97201

Electrogenic glutamate transporters have been characterized previously in photoreceptors and in glial cells isolated from the salamander retina. The glutamate-evoked current in cone cells exhibits many of the properties of the prototypic glial cell glutamate transporter (i.e., sodium-dependence and similar pharmacology) but differs in that much of the cone cell current appears to be carried by chloride ions. To investigate the molecular basis of this difference and to characterize the properties and distribution of subtypes expressed in this model system, we have isolated glutamate transporter cDNAs from salamander retina. Evidence for the expression of seven distinct subtypes was obtained by nucleotide sequencing of partial cDNAs isolated with polymerase chain reaction. Four different functional glutamate transporters subtypes, termed SR6, SR24, SR27, and SR30, were subsequently isolated from a salamander retina cDNA library. These transporters exhibit structural similarities with human glutamate transporters (J. Neurosci. 14: 5559,1994); SR6 has 92% sequence similarity to EAAT1, SR24 and SR27 have 92% and 85% similarity to EAAT2, and SR30 is a novel subtype with no reported mammalian counterpart. Using two electrode voltage clamp recordings from *Xenopus* oocytes expressing synthetic RNAs, we find that each subtype exhibits sodium-dependent inward currents in response to application of excitatory amino acids. SR24 and SR27 are inhibited by low micromolar kainate. We are currently determining the localization of these transporters in the retina by *in situ* hybridization and immunocytochemistry.

730.26

ION FLUXES THROUGH EXCITATORY AMINO ACID TRANSPORTERS J.I. Wadiche, E.W. McCleskey*, S.G. Amara, and M.P. Kavanaugh, Vollum Institute, OHSU Portland, OR 97201.

Currents and uptake of radiolabeled excitatory amino acids mediated by cloned human excitatory amino acids transporters (EAAT 1-3) were measured under voltage clamp in *Xenopus* oocytes. Consistent with a model predicting translocation of net positive charge with each transport cycle, superfusion of the transport substrate D-Asp induced inward currents in oocytes expressing EAAT2 which did not reverse at potentials up to +80mV. In contrast, D-Asp induced currents which reversed in oocytes expressing EAAT1 (E_{rev} = +9.3 ± 0.7 mV; n=46) and EAAT3 (E_{rev} = +38.0 ± 2.7 mV; n=28). The reversal potentials of the EAAT1 and EAAT3 currents shifted 54.1 ± 1.8 (n=5) and 53.7 ± 4.3 mV (n=5) per 10-fold change in $[Cl^-]_{out}$. The outward currents were abolished upon substitution of extracellular chloride with gluconate. The component of the current carried by chloride was resolved by subtraction of currents recorded before and after depletion of internal chloride andexternal chloride; this current reversed near E_{Cl}. For EAAT1, the quantity of charge translocated per molecule of [^{3}H]D-Asp varied as a function of membrane potential (from +3.5 e_0 at -100mV to -2.5 e_0 at +25mV) and was approximately +1 at E_{Cl} or when chloride ions were absent. The uptake of [^{3}H]D-Asp was not thermodynamically coupled to the chloride electrochemical gradient. The selectivity sequence of the excitatory amino acid-activated conductance was NO_3^->I^->Br^->Cl^->F^->>gluconate$^-$. The results suggest that the net current flowing through the transporter reflects translocation of one net positive charge per transport cycle plus a second current resulting from activation of a gated channel-like activity. This novel behavior provides a potential mechanism for the transporters to dampen cell excitability in addition to removal of transmitter.

730.27

POSSIBLE REGULATION OF GLUTAMATE UPTAKE BY SECOND MESSENGERS J.G. Ortiz*, O. Claudio, G. Santiago, M. L. Cordero, and J. Nieves, Dept. of Pharmacology, Univ. of Puerto Rico Medical School, PO Box 365067, San Juan, **Puerto Rico** 00936-5067

Glutamate (**GLU**) reuptake is the primary mechanism for its removal from the synapse. Several GLU transporters have been cloned. Glycosylation and possibly protein kinase C (PKC) appear as a common feature of the cloned transporters (Kanai et al., 1993). GLU uptake is increased in whole mouse brain synaptosomes preincubated with NO synthase inhibitors aminoguanidine or nitroarginine, implying that NO inhibits GLU uptake. Arachidonic acid also inhibits GLU uptake. Pertussis toxin, a G_i-protein inhibitor markedly reduces GLU uptake, while 1.0 mM Li^+, possibly by inhibiting phophatidylinositol turnover, potentiates it. These observations suggest possible regulation of GLU uptake by second messengers. Many of these effects are not observed in synaptosomes from mice with genetic audiogenic seizures, pointing to the possible relevance of these observations to experimental epilepsy. (Supported by the NIH/MBRS, RCMI and CIDIC Institutional Programs and Animal Resource Center).

730.28

PHARMACOLOGY AND REGULATION OF THE RAT SODIUM-DEPENDENT GLUTAMATE TRANSPORTER, EAAC1. L.A. Dowd,*[1] A.J. Coyle,[1] J.D. Rothstein,[2] and M.B. Robinson[1]. [1]Children's Seashore House, Depts. of Peds and Pharm., Univ. of Penn., Philadelphia, PA 19104; [2]Dept. of Neurology, The Johns Hopkins Univ., Baltimore, MD 21287.

Recently, three distinct cDNAs (EAAC1, GLT1, and GLAST) encoding SDHA Glu transporters have been isolated but these transporters do not fully reconstitute the pharmacology of transport observed in brain tissue. The first purpose of the present investigation was to determine if EAAC1 reconstitutes the properties of transport observed in synaptosomes, C6 glioma, or primary astrocyte-enriched cultures. Glu transport into EAAC1-injected oocytes and C6 glioma have similar affinities for Glu and sodium, and these values markedly differ from those observed in rat synaptosomes. Several excitatory amino acid (EAA) analogs were tested as inhibitors of L-[^{3}H]-Glu transport in oocytes expressing EAAC1 cRNA. Although EAAC1-mediated transport and cortical synaptosomal transport have similar pharmacological profiles, five EAA analogs were at least 3-fold more potent as inhibitors of cortical synaptosomal transport than as inhibitors of EAAC1-mediated transport. In contrast, all of the compounds examined inhibit transport observed in C6 glioma with similar potency to that observed in oocytes injected with EAAC1 cRNA. Consistent with these data, C6 glioma express EAAC1-like immunoreactivity. The molecular weight of EAAC1 in C6 glioma is, however, 7-10 kDa larger than the protein recognized in crude cortical synaptosomes. At present it is unclear if this difference in glycosylation contributes to the pharmacological differences observed in C6 glioma and synaptosomal membranes. Activation of PKC by TPA caused a 2.5-fold increase in EAAC1 Glu transport and this increase was due to an increase in V_{max} with no change in K_m. This increase in activity was observed within 2 minutes and remained elevated for at least 60 minutes. (NS29868)

SECOND MESSENGERS: G-PROTEINS

731.1

DESENSITIZATION OF A CATIONIC CONDUCTANCE ACTIVATED BY METABOTROPIC GLUTAMATERGIC AND MUSCARINIC RECEPTORS IN CA3 NEURONS IN VITRO: ROLE OF G-PROTEINS. N.C. Guérineau, J.-L. Bossu, B.H. Gähwiler and U. Gerber*

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Desensitization of responses mediated by activation of metabotropic receptors is a commonly observed phenomenon. In the case of the cationic conductance activated by bath-application of 1S,3R-ACPD or MCh, the desensitization is characterized by a decrease of 50 % in the current amplitude when the agonist is reapplied 3 minutes after the initial application. This desensitization occurs at the second messenger level rather than at the receptor, since the second response to agonist is still reduced when neurons were exposed first to 1S,3R-ACPD and second to MCh, or vice versa. Interestingly, the desensitization appears to be related to activation of a G-protein. In the presence of GTPγS (500 μM, non-hydrolyzable analogue of GTP) in the patch-pipette, the first application of 1S,3R-ACPD or MCh is already desensitized (50% of the amplitude of control cells). By contrast, intracellular dialysis with high concentration of GDPβS (1 mM, non-hydrolyzable analogue of GDP) inhibits the desensitization process such that the cationic current amplitude is greatly enhanced (~200 % of the control response) and in most cells persists without returning to baseline. In further experiments, we show that the G-protein involved in the desensitization is pertussis toxin-insensitive. We are now attempting to determine whether this G-protein is directly linked to the cationic channel or is coupled to subtypes of mGluRs and muscarinic receptors different from those responsible for the cationic current.

731.2

SIGNALLING PATHWAYS OF THE DOPAMINE D2S RECEPTOR: DISSECTION USING PERTUSSIS TOXIN-INSENSITIVE G PROTEIN MUTANTS M.H.Ghahremani* and P.R.Albert, Department of Pharmacology and Therapeutics, McGill University, Montreal ,Canada H3G 1Y6.

The dopamine D2S receptor couples to multiple signalling pathways through pertussis toxin (PTX) sensitive heterotrimeric guanine nucleotide binding proteins ($G_{i/o}$). When transfected in Ltk- fibroblast cells (LD2S), the dopamine D2S receptor decreases forskolin-stimulated cAMP levels and increases both PI turnover and calcium mobilization (Liu *et al.*, 1992), actions which were blocked by PTX pretreatment. In order to investigate the different subtypes of $G_{i/o}$ that mediate D2S signalling, mutation of the G_i/G_o proteins which render them insensitive to PTX treatment were made. The C-terminal Cys residue in the α subunit of $G_{i/o}$ proteins (α_o, α_{i1}, α_{i2} and α_{i3}) was converted to Ser by site-directed mutagenesis. The mutant G proteins were separately transfected in LD2S cells and clones were detected by Northern analysis. The amount of protein expressed in each clones was determined by Western Blot using antibodies against G_o or $G_{i1,2}$. In non-transfected cells G_{i2} was found to be the dominant $G_{i/o}$ protein expressed endogenously at both mRNA and protein level; G_o was at the limit of detection. Clones transfected stably with mutant G proteins showed levels of specific mRNA and protein expression which varied from one to several-fold endogenous levels of G protein. To study the role of these G proteins in signalling of the D2S receptor, $[Ca^{++}]_i$ was measured by using Fura-2 in clones expressing mutant G proteins. Clones that express amounts of mutant G protein comparable to endogenous levels were treated with PTX (50 ng/ml, 16h). This eliminates the function of endogenously expressed $G_{i/o}$ which are PTX sensitive while the mutant G protein remains unaltered. Preliminary results indicate that the D2S receptor couples most strongly to G_o over G_{i1} or G_{i2} to enhance calcium mobilization. The role of G protein subtypes in receptor coupling to adenylyl cyclase and PI turnover is being examined. The mutant G proteins provide a useful tool for the investigation of G protein coupling to receptors.

731.3

ANTISENSE KNOCKOUT OF THE STIMULATORY-TYPE G-PROTEIN, GOLF, IN THE RAT BASAL GANGLIA.

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Stimulatory-type guanine nucleotide binding proteins (G-proteins) couple neurotransmitter- and hormone- activated receptors to the stimulation of adenylyl cyclase, an enzyme that catalyzes the synthesis of the intracellular second messenger, cyclic adenosine monophosphate (cAMP). We have previously shown that G-olfactory (Golf)α protein and its mRNA are selectively localized to the neural tissue of the caudate-putamen, nucleus accumbens, and olfactory tubercle. Striatonigral and striatopallidal nerve terminals appear also to contain Golfα protein. This distribution suggests that Golfα may couple the D1 dopamine and or A2a adenosine receptors found in these brain regions to adenylyl cyclase. To investigate this possibility, we have used intrastriatal antisense injections with oligonucleotides complementary to bases surrounding the initiation region of Golfα mRNA, while oligonucleotides complementary to this region in Gsα mRNA have been used as control. Oligonucleotides are 17-20 bases in length with a phosphorothioate modified backbone. Immunohistochemistry of the rat striatum at 24 and 48 hours post injection show block of Golf protein expression by Golf antisense oligonucleotides, while Western blots confirm the loss of a 43-44 kD protein, consistent with the loss of Golf. These antisense oligonucleotides will be used to investigate the functional role of Golf in the brain.

731.4

MONONUCLEAR LEUKOCYTE G-PROTEIN LEVELS AND FUNCTION IN MAJOR DEPRESSION: EFFECTS OF ANTI-DEPRESSANT TREATMENT. L. T. Young*, P. P. Li and J. J. Warsh. Dept. Psychiatry, McMaster Univ. Hamilton, ON, L8N 3Z5 and Clarke Instit. Psychiatry, Toronto, ON, Canada.

The molecular pharmacology of antidepressants (ADs) suggests their site of action may be in the β-adrenoceptor-G-protein-adenylyl cyclase pathway. In the present study levels of G-proteins which regulate adenylyl cyclase (AC) activity and function were measured in mononuclear leukocytes (MNLs) obtained before and after 5 weeks of AD treatment in 12 subjects with major depressive disorder (MDD). MNLs were obtained from drug-free (>10 days) outpatients meeting RDC criteria for MDD and a Ham-D score >16. G-protein (α_s and α_i) levels were determined by immunoblotting and AC (basal, GTPγS-stimulated 100 μM, Forskolin-stimulated 10 μM) were determined with a radioimmunoassay. There were no pretreatment differences in either G-protein levels or functionality in patients compared with controls. Responders (1.83±0.26 pmol/mg/min, N=6) but not nonresponders (3.06±0.52, N=6) showed lower basal AC activity compared with controls (2.45±0.33, N=12, T=2.73, df16, $p<0.01$). Furthermore, there were no significant differences pre- and post- AD treatment in α_s or α_i levels or in GTPγS- or forskolin-stimulated AC activity in the depressed patients as a whole or when subgrouped into responders and nonresponders. These data do not support a G-protein mediated action for AD in the treatment of MDD. Desensitization of the AC signalling pathway may be present in treatment responders as suggested previously.

731.5

CORTICOSTERONE ALTERS G PROTEINS IN THE HIPPOCAMPUS.

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The highest concentration of glucocorticoid receptors is located in the hippocampus, an area of the brain associated with memory and emotions. Several laboratories have demonstrated that the glucocorticoid corticosterone (CT) alters the response of several neurotransmitter receptors in the hippocampus that are linked to several types of G proteins. We used Western blot analysis and immunohistochemistry to determine the effects of CT on G protein α-subunit (G_s, $G_{i1\ and\ 2}$ and G_o) levels and distribution in the hippocampus. Four treatment groups were used in our investigation: SHAM, ADX (bilateral adrenalectomy); BS (ADX + 12.5 mg CT pellet to produce basal plasma CT levels); HCT (ADX + 300 mg CT pellet to produce stress levels of CT in the plasma). Treatment was for 2 weeks. In Western blots ADX had no effect on G protein levels when compared to SHAM animals. BS increased G_s levels while HCT increased the levels of G_s, $G_{i1\ and\ 2}$ and G_o when compared to SHAM. In immunohistochemistry experiments, BS increased G_s levels in subfield CA3 when compared to ADX. BS increased G_o levels in CA1 when compared to SHAM. BS also increased G_o levels in the dentate when compared to ADX, SHAM, and CT. We conclude that 1) CT does not alter the intracellular distribution of G proteins in the hippocampus, 2) CT alters the G protein levels in the hippocampus and 3) changes in G protein levels may be a compensatory response. Supported by MH-00880 and NS-28512 to S.G.B. and BRSG784 to N.A.M. and S.G.B.

731.6

DIFFERENTIAL DOWNREGULATION OF MORPHINE AND MORPHINE-6-β-GLUCURONIDE ANALGESIA IN THE RAT BY MOR-1 AND G-PROTEIN ANTISENSE OLIGODEOXYNUCLEOTIDES. Rossi, G.C.*, Standifer, K.M., and Pasternak, G.W., Cotzias Laboratory of Neuro-Oncology, Memorial Sloan Kettering Cancer Center and Department of Pharmacology and Neurology, N Y, NY 10021.

Opioid actions are mediated through G-protein coupled receptors. The recent cloning of the four families of opioid receptors (μ, δ, κ_1 and κ_3) has confirmed their place in the seven transmembrane superfamily. Raffa et al (1994) showed that antisense oligodeoxynucleotides (ODN's) to $G_i\alpha 2$, but not $G_i\alpha 1$ or $G_i\alpha 3$ decreased morphine analgesia in mice. Using this strategy and a strategy developed against δ and κ_1 opioid receptors, we examined an antisense sequence directed against the 5' non-coding region of the MOR-1 clone and three G-protein ODN's ($G_i\alpha 1$, $G_i\alpha 2$ and $G_o\alpha$). Rats were injected into the periaqueductal gray with MOR-1 antisense or it's mismatch (4 bases changed) on Days 1, 3, and 5. Morphine, DADLE or morphine-6-glucuronide (M6-G) analgesia was then assessed after 24 hours. Other rats were injected with antisense ODN's to $G_i\alpha 1$, $G_i\alpha 2$ or $G_o\alpha$. Thirty-eight to fourty-four hours after the antisense treatment (25 μg) rats were given morphine (2.5 μg) or M6-G (18 ng) and tested for analgesia. Morphine antinociception was significantly reduced by $G_i\alpha 2$ antisense, and the MOR-1 antisense ODN. In contrast, M6-G antinociception was blocked by $G_i\alpha 1$ antisense, but not the MOR-1 antisense ODN. Both morphine and M6-G induced analgesia were unaffected by administration of $G_o\alpha$ antisense. DADLE analgesia was also significantly reduced by the MOR-1 antisense sequence. Over a period of 4-5 additional days, the animal's analgesic state returned to those values consistent with that of controls. These observations raise the possibility that M6-G, a morphine metabolite, may be mediated through an analgesic system distinct from that of two other mu_1 agonists, morphine and DADLE.

731.7

MORPHINE- (μ), NALBZOH- (κ_3), AND MORPHINE-6β-GLUCURONIDE-(M6G) MEDIATED ANALGESIC SYSTEMS ARE DIFFERENTIALLY BLOCKED BY ANTISENSE DNA DIRECTED AGAINST DISTINCT G-PROTEIN α SUBUNITS. Standifer, K.M.*, Rossi, G.C[1], and Pasternak, G.W. Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Opioids mediate analgesia through distinct spinal and supraspinal pathways, each pathway containing at least one μ, δ, and κ receptor subtype. Opioid receptor activity is pertussis-toxin sensitive, indicating G-protein α subunit involvement. Recently, several groups have tried to determine those G-protein α subunits responsible for mediating opioid analgesia. Administration of antisense DNA directed against $G_i\alpha 2$, but not $G_i\alpha 1$ or $G_i\alpha 3$, reduced supraspinal morphine analgesia in mice tested 18-24 hr later (Raffa *et al.*, 1994, *Eur. J. Pharmacol.*, **258**, p.R5). We now present data indicating μ-, κ_3-, and M6G-mediated analgesic systems show distinct sensitivity profiles to antisense directed against various G-protein α subunits. Mice (male, CD-1, 25-30 g) were injected with antisense to $G_i\alpha 1$, $G_i\alpha 2$, $G_i\alpha 3$ or $G_o\alpha$ (5 μg, i.c.v.). The effects of this supraspinal antisense DNA treatment on morphine- (700 ng, i.c.v.), M6G - (20 ng, i.c.v.) or NalBzoH- (15 μg, i.c.v.) mediated analgesia was assessed using the radiant heat tailflick assay. NalBzoH-induced analgesia was completely blocked 24 hours after administration of $G_i\alpha 1$, $G_i\alpha 3$ or $G_o\alpha$ antisense DNA ($p<0.01$), but was unaffected by $G_i\alpha 2$ antisense DNA treatment. Morphine- and M6G-mediated analgesia were reduced only by administration of antisense DNA directed against $G_i\alpha 2$ ($p<0.01$) or $G_i\alpha 1$ ($p<0.05$), respectively. These results are consistent with the existence of distinct μ- and κ_3-mediated analgesic systems (as well as distinct morphine- and M6G-induced antinociceptive systems), and underscores the complexity of opioid-mediated antinociception.

731.8

EFFECT OF CHRONIC ADMINISTRATION OF ANTIDEPRESSANTS ON THE LEVELS OF VARIOUS SUBTYPES OF G-PROTEINS ($G_s\alpha$, $G_i\alpha$, $G_{q/11}\alpha$) IN RAT BRAIN. Y. Dwivedi*, S.C. Pandey, and G.N. Pandey. Dept. of Psychiatry, University of Illinois at Chicago, IL 60612.

Antidepressants have been shown to cause changes in neurotransmitter receptors and second messenger systems. Several receptors are coupled to effector proteins through G proteins. In order to examine the role of G proteins in the mechanism of action of antidepressants and anti-anxiety drugs, we studied the effect of these drugs on the levels of expressed $G_s\alpha$, $G_i\alpha$, and $G_{q/11}\alpha$ subunits in rat cortex and hippocampus. Male Sprague-Dawley rats were given i.p. injections of desipramine (10 mg/kg), lithium chloride (2 meq/kg, twice) phenelzine (10 mg/kg), alprazolam (10 mg/kg), buspirone (10 mg/kg) and mCPP (10 mg/kg) daily for 15 days. The rats were sacrificed 24 hrs. after the last injection. The levels of expressed $G_s\alpha$, $G_i\alpha$ and $G_{q/11}\alpha$ were quantified by Western blotting using specific antibodies in cortices and hippocampi of rats treated with different drugs. We observed that lithium caused a significant decrease in the $G_i\alpha$ subunit (36%) in both cortex and hippocampus, while immunolabelling of $G_s\alpha$ and $G_i\alpha$ remained unchanged. There was no significant change in the levels of $G_s\alpha$, $G_i\alpha$ and $G_{q/11}\alpha$ proteins in cortex or hippocampus after desipramine and phenelzine treatment. We did not observe significant changes in immunolabelling of any of the G protein subunits after treatment with alprazolam, buspirone, or mCPP. However, the immunolabelling of $G_i\alpha$ protein in cortex after buspirone treatment tended to decrease and $G_s\alpha$ protein in cortex of desipramine-treated rats tended to increase, although it did not reach a statistically significant level. These results suggest that $G_i\alpha$ protein may be related to the mechanism of action of lithium.

731.9

ANTISENSE CONSTRUCTS DIRECTED AGAINST $G_{\alpha q/11}$ REDUCE MUSCARINIC M-CURRENT INHIBITION AFTER INTRANUCLEAR INJECTION IN RAT SYMPATHETIC NEURONS F.C. Abogadie, D.A. Brown, M.P. Caulfield, J.E. Haley, Y. Vallis, and N.J. Buckley*. Wellcome Laboratory for Molecular Pharmacology, University College London, Gower St, London WC1E 6BT, UK.

Our previous studies with injections of antisera against the C-terminal decapeptide of G-proteins established that $G_{\alpha q}$ and/or $G_{\alpha 11}$ are involved in the transduction of M-current inhibition by muscarinic receptor agonists (Caulfield et al. (1994), J.Physiol., 477,415). Such antisera do not discriminate functionally between $G_{\alpha q}$ and $G_{\alpha 11}$, so we have explored the use of constructs expressing sequences antisense to parts of G-protein gene sequences, as more specific tools to define G-protein involvement in muscarinic receptor modulation of the M-current.

Intranuclear injection of rat cultured superior cervical ganglion neurons was achieved using a variant of our method for injection of antisera; we verified injection by inclusion of fluorescein-isothiocyanate labelled dextran, which also enabled identification of injected cells 2 days later. In a parallel series of experiments, we established that cells injected in a similar manner with a cytomegalovirus promoter-driven β-galactosidase expressed functional protein 2 days after injection. Two days after injection with the "antisense" construct, cells had highly significant reductions in immunocytochemically-detectable Gαq/11. This was accompanied by a reduction in M-current inhibition by oxotremorine-M (1μM) from 64%±9.2% ("sense"-injected cells) to 28.8%±4% in antisense-injected neurons (n=4). These data encourage us to believe that antisense-expressing constructs will be useful tools for defining the functions of proteins involved in signal transduction in primary neurons.

731.10

CHRONIC ANTIDEPRESSANT TREATMENT INCREASES COUPLING BETWEEN THE G PROTEIN, Gs AND TYPE VI ADENYLYL CYCLASE.
J. Chen, K. Chaney and M. M. Rasenick*. Dept. of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60612

Earlier studies from this lab have suggested that chronic antidepressant treatment (electroconvulsive shock, tricyclic and atypical compounds) increases the coupling between Gsα and adenylyl cyclase, without changing the intrinsic activity of these proteins. More recently, these studies were repeated on cultured C6 glioma cells where it was demonstrated that chronic treatment with several antidepressant drugs showed a similar augmentation of GppNHp-stimulated adenylyl cyclase without altering the intrinsic activity of that enzyme (J. Neurochem, 64:724-732, 1995). These results suggested that antidepressants had a direct post-synaptic effect, and that the target of antidepressant action was some component of the membrane or cytoskeleton which regulated coupling between Gsα and adenylyl cyclase. Rats were treated chronically with ECS, amitriptyline, desipramine or amphetamine and membranes were prepared from cerebral cortex. The ability of Gsα to stimulate adenylyl cyclase was enhanced in all but the amphetamine group. Western blotting with a battery of antibodies against Gα or Gβγ showed no change in the expression of these proteins. Immunoprecipitation of membrane extracts with an anti-Gsα antibody showed that, despite no change in Gs expression, about 50% more adenylyl cyclase activity was precipitated from the antidepressant treated animals. In order to probe the nature of the increased coupling between Gsα and adenylyl cyclase, various cells were treated with antidepressants. While cells displaying Ca^{2+}-inhibited adenylyl cyclase (C6, SK-N-SH) showed increased activation of that enzyme by GppNHp or forskolin after antidepressant treatment, cells without Ca^{2+} inhibited adenylyl cyclase (COS-1, HEK 293) showed no changes in enzyme activity. This was investigated further by treating HEK 293 cells which expressed type VI adenylyl cyclase (from J. Krupinski) with amitryptiline. In the type VI-expressing cells (but not the controls), chronic antidepressant treatment increased GppNHp-activated adenylyl cyclase. Thus, it is possible that Gα interactions with type VI adenylyl cyclase are enhanced preferentially by chronic antidepressant treatment.

731.11

TUBULIN REGULATES Gαq-MEDIATED PHOSPHOLIPASE $C\beta_1$ SIGNALING. J. S. Popova[1]*, S. G. Rhee[2], J. Garrison[3] and M. M. Rasenick[1]. [1]Dept. Physiol. & Biophys., Univ. Illinois at Chicago, Chicago, IL 60612, [2]Natl. Heart, Lung & Blood Inst., Natl. Inst. Health, Bethesda, MD 20892 and [3]Dept. Pharmacol., Univ. Virginia, Charlottesville, VA 22908.

Coimmunoprecipitation and autoradiography studies have revealed that the cytoskeletal protein tubulin, with the hydrolysis-resistant photoaffinity probe [^{32}P]AAGTP (azidoanilido GTP) bound, interacts with the G protein Gαq and thus, is a potential candidate for regulation of phospholipase $C\beta_1$ ($PLC\beta_1$) activity. To study this phenomenon further, we infected Sf9 cells with recombinant baculoviruses encoding Gαq and $PLC\beta_1$ and compared the effects of GppNHp and tubulin-GppNHp (concentration-response) on membrane-associated $PLC\beta_1$ activity using exogenous [^{3}H]PIP_2 as a substrate. GppNHp stimulated the high basal $PLC\beta_1$ activity in a concentration-dependent manner. Under the same conditions, the higher concentrations of tubulin-GppNHp (500nM to 5 μM) inhibited the enzyme. In order to study the possibility that tubulin has an effect on receptor-dependent stimulation of $PLC\beta_1$ as well, we infected Sf9 cells, simultaneously, with baculoviruses bearing M_1 muscarinic receptor (E. Ross, Dallas), Gαq or $PLC\beta_1$ cDNAs. The coexpression of the three different cDNAs was verified by Western Blotting. Receptor binding studies using [^{3}H]QNB as a ligand revealed 240 fmol/mg membrane protein of expressed M_1 muscarinic receptors with an apparent Kd of 0.162 nM. Carbachol (at 1 mM) increased the transfer of [^{32}P]AAGTP from tubulin (at 1 μM) to membrane Gαq. Carbachol-stimulated $PLC\beta_1$ activity was increased in a concentration-dependent and saturable manner by GppNHp. Furthermore, carbachol (at 1 mM) appeared to blunt the inhibition of $PLC\beta_1$ by tubulin-GppNHp. Since calcium is known to increase microtubule depolymerization, these studies suggest a potential role for tubulin in the regulation of phosphatidylinositol/calcium signaling within the cell.

731.12

IDENTIFICATION OF THE G PROTEIN DOMAIN RESPONSIBLE FOR TUBULIN-ACTIVATED SIGNAL TRANSDUCTION. S. Roychowdhury*, N. Skiba, A. Greene, A. Mathew, H. Hamm and M. M. Rasenick. Department of Physiology & Biophysics, U. Illinois College of Medicine, Chicago, IL 60612

Evidence from our laboratory suggests that the cytoskeletal protein tubulin, modifies neurotransmitter receptor signaling, activating certain G proteins (Gs, Gi1 and Gq) by transferring GTP to them. In order to establish the physiological role of tubulin -Gα interaction , it was necessary to determine the interacting domains on the molecules. Initial proteolytic and chemical cleavage studies suggested a common sequence for tubulin binding (NNR/KW) exists in Gαs (272-275) and Gαi1 (256-259). Transducin lacks the above sequence and it does not interact with tubulin. To confirm the above data and to determine the relevence of the domain for functional interaction with tubulin, a number of transducin-Gαi1 chimeras were made, expressed in E. coli and purified. Restriction enzyme sites were introduced with PCR in the pHis6Gαi1 expression vector (obtained from Maurine Linder) and either Gαi or transducin sequences were filled in. These chimeras were tested for their ability to bind to tubulin by dot blot and tubulin overlay studies. Functional interaction of the chimeras with tubulin were studied by measuring the transfer of AAGTP from tubulin to chimeras and stabilization of bound nucleotide. In one chimera , a small region of Gαi1 (220-299) in transducin sequence was sufficient to allow activation of the molecule by tubulin. In a second chimera, where the region of Gi between 243-271 was replaced by transducin, activation by tubulin did not occur. Consistent with proteolytic studies, the first chimera bound tubulin with similar affinity as rGαi1 and rGαs. The second chimera did not bind tubulin. A synthetic peptide corresponding to the suspected tubulin-binding region of Gαi1 blocked the tubulin-AAGTP activation of Gαi1. The result suggests that the region NNKW in Gαi1 is important for binding and activation by tubulin. It is hoped that a synthetic peptide which blocks tubulin activation of Gα, will be valuable for in situ studies aimed at determining the "in vivo significance" of the tubulin activation of G proteins.

SECOND MESSENGERS IV

732.1

EFFECTS OF INTERLEUKIN-1β AND TUMOR NECROSIS FACTOR α ON PHOSPHOLIPID METABOLITES IN C6 GLIOMA CELLS. Robert S. Dotson[1]*, Charles F. Ide[1,2], and Sanda Clejan[3]. [1]Neuroscience Program, [2]Department of Cell and Molecular Biology, and [3]Department of Pathology, Tulane University School of Medicine, New Orleans, LA 70112.

Cytokines have been implicated in the regulation of astrocytic response to injury. Also, phospholipase activation is involved in the signal transduction cascades for cytokines in a number of mammalian cells types. The main goal of this work is to examine the involvement of phospholipase activation in the signalling pathways of the cytokines interleukin-1β (Il-1β) and tumor necrosis factor α (TNFα) using the C6 rat astrocytoma cell line as an *in vitro* model. Il-1β exposure initiates a biphasic increase in diacylglycerol levels, as measured by high-performance liquid chromatography. This suggests the activation of more than one phospholipase. TNFα-stimulated diacylglycerol production is also biphasic but continues for a longer time course than that for Il-1β. Both cytokines stimulate diacylglycerol production in a concentration-dependent manner. Phospholipase D hydrolyzes phosphatidyl-choline into phosphatidic acid and choline. Phosphatidic acid can be rapidly dephosphorylated to contribute to diacylglycerol levels. Both Il-1β and TNFα stimulate, in the presence of ethanol, phosphatidylethanol production, as measured by thin layer chromatography. This unambiguously indicates phospholipase D involvement. In the presence of ethanol, phospholipase D catalyzes the transphosphatidylation reaction, producing phosphatidyl-ethanol. The involvement of phospholipase D and the extended time course of diacylglycerol production suggest an interaction with protein kinase C.

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732.2

KAPPA OPIOID RECEPTOR AGONIST INHIBITION OF NE-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS: TWO POSSIBLE MECHANISMS OF ACTION. D. Paul*, L. D. Minor and N. Duan. Department of Pharmacology and Centers for Neuroscience and Alcohol and Drug Abuse, LSU Medical Center, New Orleans, LA 70112.

Kappa opioid agonists inhibit norepinephrine-stimulated phosphoinositide (PI) hydrolysis in neural tissue. Rat hippocampal slices labelled with ^{3}H-myoinositol were incubated in a lithium buffer for 60 min with 10 μM NE and various concentrations of one of the following kappa receptor agonists: U50,488, U62,066, bremazocine, BRL 52656-A, BRL 52974, BRL 53001-A or BRL 53117-A. PI hydrolysis was measured as the ratio of dpm released / dpm incorportated and analyzed as a percent of control PI hydrolysis. All of the kappa agonists dose-dependently attenuated NE-stimulated PI hydrolysis. BRL 5256-A (IC_{50} = 34 ±3.3 μM), U62,066 (40 ±7.4 μM), U50,488 = 41 ± 6.6 μM) and BRL 529974 (43 ±10.6 μM) were most potent and not significantly different from each other. Bremazocine (64 ± 25 μM) and BRL 53001-A (158 ± 78 μM) were of intermediate potency, and BRL53117-A (1420 ± 356 μM) was the least potent. The rank order of potencies for inhibition of NE-stimulated PI hydrolysis is similar to that reported for kappa receptor binding competition by these drugs. However, additional studies demonstrated that the K_i values for kappa agonist inhibition of PI hydrolysis is not significantly different than the K_i values for inhibition of [^{3}H]prazosin binding. Thus, it is possible that kappa agonist inhibition of NE-stimulated PI hydrolysis is mediated by competitive inhibition of α receptors.

732.3

GLUTAMATE RECEPTOR-MEDIATED PHOSPHOINOSITIDE TURNOVER IN BERGMANN GLIA CELLS IN CULTURE.

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Primary cultures of cerebellar Bergmann glia cells were prepared from 14-day-old chick embryos. We identified and characterized glutamate metabotropic receptors coupled to phosphoinositide turnover in these cells. Glutamic acid at a concentration of 1 mM induced an 85 % increase in [^{3}H]-inositol phosphates (InsPs) acumulation within 30 minutes in cells preincubated with [^{3}H]-myo-inositol in the presence of 10 mM lithium chloride. Glutamic acid analogues (1 mM) stimulated InsPs accumulation in the following order: L-aspartate>L-glutamate>quisqualate>N-methyl-D-aspartate>Kainate>1-Aminocyclopentane-1,3-dicarboxylate. NMDA-induced response was significant since one minute of stimulation. The potency of excitatory amino acid analogues for stimulating InsPs formation was determined within a concentration range from 10 nM to 1 mM. The L-glutamate-induced response was blocked most effectively by MK-801(5μM)> CPP(200 μM)>>DNQX(50 μM); AP5 and MCPG showed no effect. The effect of NMDA was inhibited by verapamil (10μM), which blocks T-type Ca^{2+} channels whereas nifedipine (10 μM) and dantrolene (30 μM) showed no effect. These studies provide clear evidence for the presence of glutamate receptors coupled to phosphoinositide metabolism on Bergmann glia cells, perhaps involved in the modulation of synaptic transmission in the cerebellum, through their close apposition to glutamatergic synapses in this organ. Supported by Grant 3375-N from CONACYT.

732.4

A NOVEL DIACYLGLYCEROL (DG) KINASE ISOZYME (TYPE IV) FROM RAT BRAIN RESEMBLES EYE-SPECIFIC DG KINASE OF DROSOPHILA WHICH IS ENCODED BY RETINAL DEGENERATION A GENE.

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In the process of signal transduction, (DG) kinase is thought to be involved in the resynthesis of phosphatidylinositol by converting the second messenger DG to phosphatidic acid. DG kinase is thus regarded as an attenuator of the activity of protein kinase C. Recent studies have shown that DG kinase can participate in signal transduction through not only G protein-coupled receptors but also receptor tyrosine kinase. In order to clarify the functional significance of DG kinase in various cascade, we have so far cloned three DG kinase isozymes from a rat brain cDNA library and characterized their enzymatic properties and localizations. In this study we isolated a novel cDNA for DG kinase (DGK-IV) from a rat brain cDNA library. This cDNA encoded a protein of 929 amino acids with a calculated molecular weight of104kDa. The primary structure of type IV was distinct from those of type I, II and III in that type IV contained no EF-hand motifs and four ankyrin-like repeats were attached to the carboxyl terminus. This structural feature of DG kinas-IV closely resembles the recently cloned eye-specific DG kinase of Drosophila which is encoded by retinal degeneration A gene. However, the rat mRNA for type IV was much more dominantly detected in the brain than the eye, the expression was localized in all neurons, rather intensely in the cerebral and cerebellar cortices. These morphological and structural features of DG kinase-IV suggest that DG kinase-IV belongs to a new family of DG kinase distinct from type I, II and III.

732.5

THE EFFECT OF ETHER LIPIDS ON PHOSPHATIDYLINOSITOL (PI) METABOLISM IN PRIMARY NEURONAL CULTURES OF CORTEX, HIPPOCAMPUS AND CEREBELLUM.

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Platelet activating factor (PAF) and dodecylglycerol (DDG) are structurally similar ether lipids, both exhibiting a variety of biological effects including antibacterial activity and altered differentiation of neurons and oligodendrocytes. However, PAF, unlike DDG, stimulates Ca^{++} mobilization, platelet aggregation and serotonin secretion from isolated platelets. Interestingly, both DDG and PAF stimulate acetylcholinesterase and choline acetyltransferase activity differentially in primary neuronal cultures. To continue investigation of Ca^{++} mobilization, DDG and PAF were examined for effects on PI metabolism in primary cultures from cerebral cortex, cerebellum and hippocampus. Brain regions from embryonic day 17 rat embryos were separated by gross dissection taking care to discard meninges and blood vessels. Cells were dissociated and plated in a serum containing medium. After 24 hrs, cultures were treated with cytosine arabinoside for 4 hrs, and the medium was changed to a chemically defined serum free medium. Acetylcholinesterase and choline acetyltransferase activities were measured to assess the differentiation of the neurons. PI metabolism was measured as previously described (DeCoster and Yourick, Int. J. Dev. Neurosci. 12(3):227-233, 1994) excepted incorporation and agonist incubations were increased to 2 hrs. DDG, but not PAF, increased PI metabolism 76% over basal in hippocampal cultures. PAF, but not DDG, caused a statistically significant reduction of 17% in PI metabolism in cerebellar cultures. Neither compound had any effect when compared to control in cultures from cortex. DDG and PAF have substantially different effects on PI metabolism dependent on brain regions analyzed.

732.6

ACTION OF CHOLERA TOXIN ON CARBACHOL-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN CEREBELLAR NEURONS.

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Cholera toxin (CTX) and pertussis toxin (PTX) are conventional tools used to study the role of G-proteins in receptor-effector interactions. Activation of the phosphoinositide (PI)-specific phospholipases by transmitter receptors is mediated trough PTX-sensitive $G\alpha_{o,i}$ proteins or PTX-insensitive $G\alpha_{q,z}$ proteins. So far, direct regulation of PI-specific phospholipases by G-proteins which can be ADP-ribosylated by CTX has not been observed. In primary cultures of cerebellar granule cells, the stimulation of muscarinic receptors by carbachol (Carb) results in enhanced PI hydrolysis that was inhibited by about 50% when granule cells were treated overnight with CTX (3 ug/ml). A similar treatment of cells with PTX failed to affect this Carb-induced response. The pretreatment of granule cells with CTX caused a drastic decrease of [^{32}P] labeling of a 50 kDa protein band as revealed by CTX-dependent ADP-ribosylation of granule cell homogenates in the presence of [^{32}P]NAD followed by SDS-PAGE. The half-maximal blockade of back ADP-ribosylation of this protein band appeared after 45 min, and reached 80% in 3 h. However, a 3 h preincubation of granule cells with CTX failed to decrease the Carb-induced PI hydrolysis. The 50 kDa protein band was identified by Western blot using an anti-$G\alpha_q$ antiserum. Pretreatment of granule cells with CTX (20 min to 3 h) diminished the immunostaining intensity of this protein band and led to the appearance of a new 52 kDa band. This later band was never observed if CTX was incubated in vitro with granule cell homogenate. The presence of Carb during the incubation of granule cells with CTX failed to affect the CTX-induced ADP-ribosylation. However, Carb decreased twice the intensity of immunostaining of the 50 kDa band, as compared with the effect of CTX alone. These results indicate that a short-term (less than 3 h) action of CTX on cerebellar granule cells either promotes a fast degradation and/or modification of $G\alpha_q$, or inhibits its biosynthesis. However, these changes are not responsible for the inhibition of Carb-stimulated PI hydrolysis. In contrast, a long-term (overnight) treatment of granule cells with CTX results in the uncoupling of muscarinic receptors from PI hydrolysis through mechanisms which are not directly related to the ability of CTX to ADP-ribosylate $G\alpha_q$.

732.7

ENDOTHELIN STIMULATES THE HYDROLYSIS OF PHOSPHATIDYLCHOLINE BY PHOSPHOLIPASE D IN MOUSE STRIATAL ASTROCYTES.

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In striatal astrocytes, receptors for endothelins (ETs) are associated with several intracellular signaling pathways: ETs induce a sustained influx of calcium, inhibit adenylate cyclase activity and markedly activate phospholipases C and A2 (Marin et al., *J. Neurochem.* 56:1270-1275, 1991; Tencé et al., *Eur. J. Neurosci.* 4:993-999, 1992). We report here that ET-1 and ET-3 also stimulate a phospholipase D (PLD) activity which hydrolyses phosphatidylcholine into phosphatidic acid (PA).

In primary cultures of mouse astrocytes prelabelled with tritiated myristic acid, ET-1 (0.1 μM) induced a sustained production of both tritiated PA and diacylglycerol (DAG). This effect resulted from the activation of a PLD since in the presence of ethanol, ET-1 and ET-3 also stimulated time- and dose-dependently the formation of tritiated phosphatidylethanol (EC50: 2-4 nM). The ET-1 activation of PLD required extracellular calcium and involved a pertussis toxin-sensitive G-protein. Protein kinase C seemed to play a role in this activation since inhibitors of this enzyme, as well as a prolonged pretreatment with a phorbol ester (PMA), substantially reduced the formation of phosphatidylethanol. PMA also stimulated PLD activity. However, this effect was additive with that of ET-1 and furthermore it did not require extracellular calcium. These results suggest that at least two distinct mechanisms are involved in the control of PA formation in striatal astrocytes.

The stimulation of PLD by ETs could have particular physiological consequences. Indeed, a sustained production of PA and of its derivative DAG, the physiological activator of protein kinase C, could account for the mitogenic effects of ETs on astrocytes.

732.8

IN UTERO ISCHEMIA ENHANCES QUISQUALATE-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IN RAT HIPPOCAMPAL CELL CULTURES.

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Hypoxic-ischemic (HI) insult in perinatal life is a major cause of neuronal injury and impaired postnatal development. Effects of intrauterine HI on quisqualate (QA)-stimulated phosphoinositide (PPI) hydrolysis were studied in rat hippocampal cell cultures prepared from 19 day fetal rats which experienced an HI insult on G15. On gestation day 15, ischemia conditions were achieved by complete clamping of the uterine blood vessels for 30 min followed by removal of the clamps to permit reperfusion. Sham operation (surgery without vasculature clamping) was conducted in the control groups. The uterine horns were returned to the dam's abdomen after surgery and hippocampal neuronal cultures were prepared from the G19 fetuses. At time 14 days in vitro, cell density and protein content of the cultures were not significantly different between the sham-operated and HI groups. Cells were labeled with [^{3}H]-myoinositol (2 μCi per well) 24 hr before they were stimulated with quisqualate (100 μM) in the presence of 10 mM LiCl. The formation of labeled inositol monophosphate was measured as a representative of receptor-stimulated PPI hydrolysis. Intrauterine HI did not affect the incorporation of radioactive inositol into the cell membrane nor the basal level of PPI hydrolysis (3125 and 3051 dpm/mg protein for the HI and the sham groups, respectively). Stimulation with QA induced a 4.6-fold increase in PPI hydrolysis over the basal level in HI cells, whereas this increase in the sham groups was only 3.6-fold. PPI hydrolysis stimulated by carbachol was not affected by intrauterine HI insult. These data suggest that intrauterine HI may have long-lasing effects on glutamate metabotropic receptor-mediated signal transduction in hippocampal cells.

732.9

CALCIUM, GTP AND CARBACHOL STIMULATION OF HIPPOCAMPAL PHOSPHOINOSITIDE HYDROLYSIS FOLLOWING CHOLINERGIC DENERVATION AND SYMPATHETIC INGROWTH.
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Decreases in central cholinergic activity accompanied by increases in norepinephrine (NE) concentration have been described in Alzheimer's patients. To determine how these changes might affect brain biochemistry, our laboratory has utilized the model of hippocampal sympathetic ingrowth, in which peripheral sympathetic fibers, originating from the superior cervical ganglia, grow into the hippocampus following cholinergic denervation via medial septal lesions. Since sympathetic ingrowth can be prevented by ganglionectomy (Gx), the effects of cholinergic denervation alone (CD; MS lesions + Gx) can clearly be separated from the effects of hippocampal sympathetic ingrowth (HSI; MS lesion+sham Gx). HSI and CD have been found to differentially affect cholinergically stimulated phosphoinositide hydrolysis (PI) and the affinity of muscarinic receptors (mAChR) in a manner suggesting an alteration in coupling efficiency between the mAChR and PI turnover. To test this hypothesis, we have utilized newly developed methods in which prelabeled phosphoinositides (i.e., [^{3}H]-PI) are added to hippocampal membranes and hydrolysis assessed after the addition of calcium, to activate phospholipase C (PLC), GTPγS, to activate G-proteins, or GTPγS + carbachol, to activate the entire receptor complex. PLC activity in dorsal and ventral hippocampus was similar in HSI, CD and control animals. However, GTPγS stimulated PI hydrolysis in dorsal hippocampus was found to be significantly decreased in CD, when compared to control and HSI, animals ($p<0.02$), with HSI increasing PI turnover by 27% to control levels. Carbachol+GTPγS stimulated PI hydrolysis in dorsal hippocampus was also found to be decreased in CD when compared to control and HSI animals ($p<0.004$), with HSI increasing PI turnover by 15% to control levels. The results suggest that both sympathetic ingrowth and cholinergic denervation alone alter G-protein function, which may be the mechanism by which HSI and CD induce changes in mAChR affinity and cytosolic PI turnover.

732.10

SIGMA RECEPTOR REGULATION OF NMDA-STIMULATED [^{3}H]ARACHIDONIC ACID RELEASE FROM CEREBELLAR GRANULE CELLS IS PERTUSSIS TOXIN-SENSITIVE.
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We have previously reported that sigma (σ) receptors are involved in the regulation of arachidonic acid release from cerebellar granule cells. We have now investigated the effects of the sigma agonists (+)pentazocine and BD737 on NMDA-stimulated [^{3}H]arachidonic acid ([^{3}H]AA) release from cerebellar granule cells in the absence and presence of pertussis toxin.

Cerebella of eight-day old neonatal rats were dissected and chopped into pieces. Cells were mechanically and enzymatically dissociated, subjected to differential centrifugation, resuspended and plated on polyethyleneimine coated dishes. Cell cultures were treated with 10 μM cytosine arabinofuranoside to prohibit growth of non-neuronal cells. After at least eight days in culture, cells were incubated with 1 μCi/dish [^{3}H]AA for 24 hrs ± pertussis toxin (0.1 nM), then washed to remove residual, unaccumulated labeled eicosanoid. Release of [^{3}H]AA was stimulated by a 10 min exposure to 50 μM NMDA in the presence or absence of a sigma drug.

Increasing concentrations of sigma agonists (+)pentazocine and BD737 completely inhibited NMDA-stimulated [^{3}H]AA release from cerebellar granule cells. Furthermore, the sigma$_1$ antagonist DuP 734 reversed the inhibition of [^{3}H]AA release by both agonists. In contrast, (+)pentazocine and BD737 failed to inhibit [^{3}H]AA release from pertussis toxin-treated cells. These data suggest that sigma receptors regulating NMDA-stimulated arachidonic acid release in cerebellar granule cells are G_i or G_o protein coupled. (Supported by a grant from NIDA to LLW and by a NIGMS predoctoral fellowship to GMG.)

732.11

CHARACTERIZATION OF PLASMALOGEN-SELECTIVE PHOSPHOLIPASE A_2 FROM BOVINE BRAIN CYTOSOL.
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The release of arachidonate from plasmalogen may be a receptor-mediated process catalyzed by 39 kDa plasmalogen-selective phospholipase A_2 (PLA_2). The purified enzyme is markedly inhibited by polyvalent anions such as phosphate, sulfate and citrate, with IC_{50} values of 400 mM, 150 mM and 5 mM, respectively, and stimulated by Triton X-100 and Tween-20. Octyl glucoside, sodium deoxycholate and taurocholate markedly inhibit enzymic activity. Non-specific inhibitors of PLA_2, such as quinacrine, nordihydroguaiaretic acid and manoalide, produce a dose-dependent inhibition. However, arachidonyl trifluoromethyl ketone (a potent inhibitor of 85 kDa cytosolic PLA_2) and bromoenol lactone (an irreversible inhibitor of 40 kDa plasmalogen-selective PLA_2 from myocardium) have no effect on enzymic activity. Finally, the 39 kDa plasmalogen-selective PLA_2 is inhibited by free fatty acids in a dose-dependent manner. Docosahexaenoate is the most potent inhibitor, followed by arachidonate, eicosapentaenoate and heptadecanoate. The inhibition by docosahexaenoate can be reversed with bovine serum albumin.

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732.12

INDUCIBLE PROSTAGLANDIN SYNTHASE AND *ZIF*-268 mRNA UPREGULATION IN VASOGENIC CEREBRAL EDEMA: INHIBITION BY A PAF ANTAGONIST.
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Cerebral hypoxia, like global brain ischemia, triggers the release of glutamate which, in excess, leads to neuronal damage. Free fatty acids and diacylglycerols also accumulate *(BBA* **218**:1-10, 1970) due to activation of phospholipases A_2 and C. Platelet-activating factor (PAF), a product of PLA_2, is a mediator of neuronal damage. Prostaglandin H synthase (PGS, cyclooxygenase) is the rate-limiting enzyme in the synthesis of PGE_2, PGF_{2F}, PGD_2, prostacyclins, and thromboxanes. The inducible prostaglandin synthase mRNA (PGS-2, TIS-10), increases rapidly in response to a wide variety of stimuli such as mitogens and inflammation. Ischemia-reperfusion, single electroshock (ECS), and brain injury increase PGS-2 message levels in nervous tissue. After cryogenic injury, a model of vasogenic brain edema, the levels of PGS-2 mRNA rose rapidly reaching a peak at 2 hours, and remained upregulated after 24 hours. Levels of TIS-8 mRNA, a zinc-finger synthesis and a transcription factor, peaked after 1 hour of the injury and returned to control levels after 6 hours. The PAF antagonist BN-50730 partially inhibited the increase in both PGS-2 and TIS-8 mRNAs. Levels of tissue edema were measured by plasma Evans Blue extravasation. The animals pretreated with BN-50730 or dexamethasone were partially protected. Thus, PAF receptors may be involved in brain damage after injury and the PAF antagonists may be useful as pharmacological tools to protect the brain from that damage. Supported by DAMD-17-93-V-3013.

732.13

ACTIVATION OF THE PLA_2-AA SIGNALING CASCADE REGULATES 5-HT_{1A} RECEPTOR FUNCTION IN CHO CELLS.
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We have reported that CHO cells naturally express a 5-HT_{1B} receptor subtype (Berg *et al*, Mol Pharmacol. 1994. vol 46: 477- 484) which is regulated by a cyclooxygenase-dependent arachidonic acid (AA) metabolite produced when phospholipase A_2 (PLA_2) is activated with melittin or the purinergic receptor agonist ATP (Berg *et al, Soc. Neurosci.* 1994. vol 20:1158). Currently, we are investigating the effect of activation of the PLA_2-AA signaling cascade on the 5-HT_{1A} receptor system in CHO cells expressing the human 5-HT_{1A} (G21) receptor (≈ 130 fmol/mg protein). The inhibition of forskolin (1 μM)-stimulated cAMP accumulation (FScA) by dipropyl-5-carboxamidotryptamine (dp-5-CT) in the presence and absence of melittin (2.5 μg/ml) or ATP (1 mM) was measured. In control experiments, both melittin and ATP increased AA release by 620% and 100% above basal, respectively, and, as we found previously, reduced 5-HT_{1B}-mediated inhibition of FScA. Melittin also reduced 5-HT_{1A} responsiveness. The EC_{50} for dp-5-CT was shifted to the right (pEC_{50} = 7.53 ± 0.06 {30 nM} vs 7.74 ± 0.05 {18 nM}; $p \leq 0.05$; n=4) and the maximal inhibition was reduced (74% ± 2% vs 83% ± 1%; $p \leq 0.01$) in the presence and absence of melittin, respectively. In contrast, ATP had no effect on 5-HT_{1A}-mediated inhibition of FScA (n=2). Thus, although the responsiveness of the 5-HT_{1A} receptor system may be regulated by the PLA_2-AA signaling cascade, the mechanism of this regulation may differ from that for the 5-HT_{1B} receptor system. (Supported in part by USPHS grants HD 26437, and MH 48125)

732.14

SPREADING DEPRESSION ELEVATES INDUCIBLE CYCLOOXYGENASE IMMUNOREACTIVITY IN DISCRETE CORTICAL REGIONS.
R.P. Kraig*, C. Breder & P. Park. Dept. Of Neurology, The University of Chicago, Chicago, IL

Eicosanoids are powerful paracrine signals which may be involved in such diverse phenomena as conduction of messages in sensory neuronal pathways and the transformation of glia into reactive species. Spreading depression (SD) is a phenomenon that can induce gliosis uncompromised by cellular necrosis. Accordingly, to begin examining how eicosanoids might influence gliosis we characterized how recurrent SD altered the immunoreactivity (ir) for the inducible form of cyclooxygenase (iCOX).

Male, Wistar rats (n=72) were anesthetized with halothane. SD was induced in parietal cortex by superfusion of potassium chloride (1.0M, 3 hrs). Micropipettes placed in both frontal cortices recorded the occurrence of SD ipsilateral to the stimulus and its absence in contralateral, control cortex. Animals were allowed to recover for 1, 3, & 6 hr; and 1, 2, 3, 7, 14, 21, & 28 days. They were then re-anesthetized and processed for iCOX-ir. Blocking studies established the specificity of immunostaining. Computer-based and semi-quantitative image analyses of the log ratio of left, experimental versus right, control cortex were done in six distinct cortical regions showing iCOX-ir: insular, motor, piriform, and perirhinal cortex as well as the lateral nucleus of the amygdala and hippocampus. SD caused a significant ("p" values varying between less than 10^{-6} to 0.05) increase in iCOX-ir from 3 hr to 3 days after SD in all brain regions except amygdala and hippocampus. The lack of significant change in these two zones may stem from a symmetric increase in iCOX-ir. Pretreatment with indomethacin and mepacrine had no affect on SD-induced iCOX-ir changes while pretreatment with dexamethasone and L-NAME reduced the rise in iCOX-ir to a nonsignificant difference between sides. Treatment with sodium nitroprusside and ephedrine also altered iCOX-ir.

These results show that neocortical SD results in a significant rise in iCOX-ir within discrete cortical regions. Furthermore, these rises in iCOX-ir can be reduced by modulation of eicosanoid and nitric oxide metabolism.

732.15

DISTRIBUTION OF CYCLOOXYGENASE ISOFORMS & NEURONAL NITRIC OXIDE SYNTHASE IN MAMMALIAN CORTEX. [1]C.D. Breder*, [2]W.R. Tracey & [1]R.P. Kraig. [1]Dept. of Neurology, The University of Chicago, Chicago, IL 60637 and [2]Central Research Division, Pfizer, Inc., Groton, CT 06340.

Inflammation is a complex reaction that includes increased production of powerful, paracrine mediators such as eicosanoids and nitric oxide (NO). NO can modulate the activity of cyclooxygenase (COX), an enzyme that produces one class of eicosanoids, prostaglandins. For such interactions to be of potential importance to CNS function, COX and NO synthase (NOS) enzymes should be regionally co-localized since diffusion distances for products of these enzymes is relatively small. Accordingly, we have begun to characterize the regional distribution of constitutive (COX1) and inducible (COX2) cyclooxygenase compared to neuronal NOS (gift from H. Schmidt, Medizinische Universitatsklinik; Wurzburg, Germany) using immunohistochemistry.

COX1 data was derived from sheep[1] and COX2[2] from rat. Blocking studies established the specificity of staining. Within neocortex, COX1 showed a dense localization to granular and infragranular cells; COX2 was densely localized to supragranular cells; and nNOS was sporadically localized to infragranular cells with many fine fibers being stained. Within allocortex, COX1 was densely localized to cells of layer 3&5 and COX2 to cells of layers 2,3,&5. In this same area, nNOS immunoreactive cells were seen in infragranular layers. Fibers positive for nNOS were densely packed in a putative terminal field in layer 2 of the agranular insular cortex and dorsolateral entorhinal cortex, a region where many COX2 cells were seen. Similar regional co-localizations of COX isoforms and nNOS were seen in hippocampus and amygdala.

These studies show that COX isoforms and nNOS co-localize to similar brain regions of cortex. Thus, products from these enzymes conceivably could interact in the CNS. Furthermore, since co-localization of these enzymes is seen in resting brain, such interactions may be an important component of normal brain function. 1. Breder, et al. **J. Comp. Neurol.**, 1993. 2. Breder et al., **J. Comp. Neurol.**, 1995.

732.16

FIVE-LIPOXYGENASE AND ITS ACTIVATING PROTEIN (FLAP) ARE EXPRESSED IN HIPPOCAMPAL NEURONS AND REQUIRED FOR SOMATOSTATIN SIGNALING. P. Schweitzer*, C.-H. Lammers[1], S.G. Madamba, G.R. Siggins and D. Piomelli[2]. Neuropharmacology, The Scripps Research Inst., La Jolla, CA 92037, [1]Max-Planck-Inst. of Psychiatry, Munich, Germany, [2]The Neuroscience Institute, San Diego, CA 92121.

The 5-lipoxygenase activating protein (FLAP) is required, in peripheral cells, for the activation of 5-lipoxygenase (5-LO) and for the resulting synthesis of leukotrienes from arachidonic acid. In the brain, leukotrienes have been implicated in several pathophysiological events and in the electrophysiological effect of somatostatin (SST) on CA1 hippocampal neurons, where the neuropeptide enhances the voltage-dependent K^+ current $I_{K(M)}$. Yet, the cellular origin and role of these messengers are still poorly understood. Therefore, we localized 5-LO and FLAP mRNA in rat brain by *in situ* hybridization and found the highest labeling densities in hippocampus and cerebellum where 5-LO and FLAP appeared to overlap. Immunostaining experiments showed that FLAP protein was associated with pyramidal neurons of CA1 to CA3 and with dentate granule cells. Moreover, most neurons in the CA1 pyramidal cell layer showed both FLAP immunostaining and 5-LO hybridization signals, confirming the co-localization of 5-LO and FLAP. Electrophysiological studies using intracellular voltage-clamp recordings in the rat hippocampal slice preparation showed that the ability of SST to augment $I_{K(M)}$ in CA1 neurons was prevented by treatment with MK-886, a potent and selective FLAP inhibitor. Leukotriene C4 overcame the blocking effect of MK-886 on $I_{K(M)}$ augmentation by SST. Furthermore, we characterized a second effect of SST that involved activation of a voltage-insensitive K^+ conductance different from $I_{K(M)}$. Our results provide strong evidence for the presence and signaling role of 5-LO and FLAP in central neurons and support a participation of the 5-LO pathway in the SST receptor transmembrane regulation of $I_{K(M)}$.

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732.17

***IN VITRO* ACTIVATION OF *LIMULUS* BRAIN AND CARDIAC PROTEIN KINASE C BY PHORBOL ESTERS AND ARACHIDONIC ACID: DEPENDENCE ON CALCIUM AND PHOSPHOLIPID.** Byron D. Ford*, Easton A. Reid and James G. Townsel. Department of Physiology, Meharry Medical College, Nashville, Tennessee 37208

Protein kinase C (PKC) is a multifunctional serine/threonine phosphorylating enzyme and plays an important role in numerous cellular functions. Twelve isoforms of PKC have been identified in mammalian tissues by molecular cloning. The isoforms are grouped into three subtypes based on co-factor requirements for activation. The activation of conventional PKC's (cPKC's) requires calcium, phosphatidylserine (PS) and diacylglycerol (DAG). Novel PKC's (nPKC's) require PS and DAG but are calcium independent. Phorbol esters (PE) can substitute for DAG in the activation of both cPKC's and nPKC's. Atypical PKC's (aPKC's) are not activated by calcium, DAG or PE but by PS and unsaturated fatty acids. Our laboratory has previously reported the presence of seven PKC isoforms in *Limulus* cardiac and neuronal tissues (Ford *et al.*, Biochem. and Biophys. Res. Comm., 1995). In addition, data from our laboratory and others have suggested a possible role for multiple PKC subtypes in the regulation of choline uptake (Saltarelli *et al.*, J. Neurosci., 1990; Ford and Townsel, Soc. Neurosci. Abs., 1994). This study demonstrates the presence of multiple PKC subtypes in *Limulus* brain and cardiac tissues based on co-factor requirements of PKC activity. The phorbol esters PMA and PDBu were both shown to activate PKC in brain and cardiac extracts. Arachidonic acid (AA) also activated PKC in both tissues. PE and AA stimulated PKC activities were additive. PE stimulated PKC activity was partially inhibited in the absence of calcium while AA stimulated PKC activity was calcium independent. Kinase activation by both PE and AA required the presence of PS. These results will aid in the elucidation of the specific roles of PKC subtypes in choline uptake. This project was supported in part by NSF grant HRD-9106096, DOE P200-A40516 and NIH grants GM08037, RR03032 and MH 19843.

732.18

LIPID METABOLISM MODIFICATION IN THE HUMAN NEUROBLASTOMA SK-N-BE DIFFERENTIATED WITH RETINOIC ACID. A.Petroni*, N. Papini, P.La Spada, M.Blasevich, and C.Galli.Institute of Pharmacological Sciences, University of Milan, via Balzaretti 9, 20133, Milan, Italy.

We have studied the effect of retinoic acid (RA) in the human neuroblastoma SK-N-BE. RA, an analogue of vitamin A, has been shown to inhibit cell growth, to change the morphology of neuroblastoma cells. RA is also an activator of peroxisome function and is possibly involved in the control of lipid homeostasis.

To investigate the degree of cell differentiation and the inhibition of cell growth by RA we have measured neurite outgrowth and labeled timidine incorporation. We have evaluate if RA could also modify lipid metabolism in SK-N-BE.The following parameters have been investigate : the fatty acids (FA) composition of cell lipids, the conversion of labeled linoleic acid (LA) to longer chain FA, the incorporation of [3H]glycerol in all major lipid classes. All the indicated parameters were evaluated in undifferentiated and RA-differentiated SK-N-BE at different time periods. RA was incubated at the concentratio 10 µM. Undifferentiated and differentiated cells were incubated with labeled LA (0.5 µCi/dish,18 hours)or glycerol (1 µCi/dish,24 hours). The cells were scraped and extracted, FA composition was evaluated by GLC. Extracted of LA labeled metabolites were injected in HPLC connected with a radiodetector on line. FA of the n-6 series: arachidonic acid and 22:4 increased, also 22:5 and 22:6 of the n-3 series was increased. The conversion of LA was enhanced at 24 hours than after 7 day differentiation. Glycerol incorporation into the main lipid classes differed according to the different incubation periods. After treatment with RA neurite outgrowth was markedly enhanced and cell growth inhibited. Our data indicate a certain modification of lipid parameters, the molecular mechanism involved in RA-differentiation will be investigated.

732.19

LYSOPHOSPHATIDIC ACID IS A PUTATIVE MESSENGER IN NEURONS. R. Diaz-Arrastia*, and E. Hashemi, Dept. of Neurology, University of Texas Southwestern Medical School, Dallas, TX 75235.

Lysophosphatidic acid (LPA) is a messenger molecule which has potent effects in a number of cell types, including neurons. Because LPA is relatively water soluble, it can diffuse from the site of production to neighboring cells, and may play a role during synaptic plasticity and in the response of neurons to injury. We have found that (1) mammalian brain contains a PLA_2 activity which preferentially hydrolyses phosphatidic acid (PA) to form LPA; (2) cultured neural cells produce LPA in response to activation of cell-surface receptors; and (3) exogenously added LPA potentiates cell death induced by glutamate in an *in-vitro* model of excitotoxicity. PA-specific PLA_2 was identified in membranes prepared from rat brain. The enzyme was solubilized using 1% cholate and 1 M KCl, and enriched several hundred-fold by gel filtration, anion-exchange, and hydroxylapatite chromatography. The enzyme does not require Ca^{2+} for activity and while selective for phospholipid polar head groups, does not discriminate between phosphatidates with different acyl chains in the *sn*-2 position. [^{3}H]-LPA production was measured in NG108-15 neuroblastoma x glioma cells which had been labelled with [^{3}H]-palmitate. Treatment of cells with ATP or thrombin resulted in 3 - 5 fold increase in levels of [^{3}H]-LPA, an effect which was mimicked by phorbol esters. There was a parallel but quantitatively greater production of [^{3}H]-PA in response to activators. Finally, the effect of exogenously added LPA on excitotoxicity was studied in cultures of NT2-N neurons which had been terminally differentiated by exposure to retinoic acid. Addition of 1 µM LPA increased tha amount of LDH released into the medium induced by subthreshold doses of glutamate (100 µM). These results suggest that LPA is a messenger in neurons which may play a role in excitotoxicity.

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732.20

ECLOSION HORMONE STIMULATES THE PRODUCTION OF CMP-PA WITH LITTLE CHANGE IN DAG LEVELS. P. J. Simpson* and D. B. Morton
ARL Division of Neurobiology and the Department of Biochemistry, University of Arizona, Tucson, AZ, 85721

Eclosion hormone (EH) is a 62 amino acid neuropeptide which triggers ecdysis behavior at the end of each molt in the tobacco hornworm, *Manduca sexta*. Stimulation of this behavior by EH is mediated by a rise in cGMP in the central nervous system prior to ecdysis. Previous work in this laboratory indicates that this rise is due to activation of a soluble guanylate cyclase (GC), perhaps through the interaction of GC with a lipid messenger. GC activation is not associated with nitric oxide (NO) production, but is blocked by a number of inhibitors of lipid metabolism, such as phospholipase C, phospholipase A_2, diacylglycerol lipase, and lipoxygenase blockers.

This study examines the action of EH on a variety of lipids in the CNS. Previous studies have shown that EH stimulates the production of inositol trisphosphate in the nervous system. Here we show that there is no, or little, evidence for a parallel increase in the levels of diacylglycerol in response to EH. There is, however, an increase in the levels of cytidine monophosphate-phosphatidic acid (CMP-PA). CMP-PA is produced downstream of DAG as part of the inositol recycling pathway. This increase is similar in both time course and EH sensitivity to the increase in cGMP and is blocked by many of the same inhibitors that block the EH-stimulated increase in cGMP.

Additionally, the fates of several other phospholipids were examined in response to EH. These phospholipids include phosphatidic acid and phosphatidylinositol as well as the major membrane phospholipids; phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. Preliminary evidence suggests that exposure of the CNS to EH results in a decrease in the incorporation of [^{32}P]-PO_4 in a number of these phospholipid species.

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733.1

DEOXYCORTICOSTERONE INCREASES NEUROPEPTIDE-mRNA LEVELS IN THE STRIATUM L. R. Lucas*†, J. A. Angulo††, and B. S. McEwen† †Lab of Neuroendocrinology, Rockefeller University and ††Dept of Biological Sciences, Hunter College CUNY, New York, NY 10021

Glucocorticoids have been shown to increase tachykinin- and enkephalin-mRNA levels in the striatum of adrenalectomized rats [Chao and McEwen (1991) Mol. Brain Res., 9, 307-311]. To investigate the effects of Type I or mineralocorticoid activation on striatal neuropeptide mRNA levels, we administered deoxycorticosterone (DOCA), an adrenal steroid with higher affinity for Type I receptors, to adrenalectomized (ADX) animals. Adult male rats were either sham operated or ADXed. After one week recovery, animals were either injected with sesame oil, corticosterone (CORT, 0.5mg/kg), or DOCA (5mg/kg), s.c., daily for 3 or 7 days and sacrificed 18h after the last injection. Neuropeptide-mRNA levels in the striatum were determined by high-resolution *in situ* hybridization histochemistry with ^{35}S-labelled riboprobes on 20μm thick coronal brain cryostat sections. After stringent washing and drying, sections were dipped in NTB-2 photographic emulsion (Kodak) and exposed for 1 to 2 weeks. Reduced silver grains were observed over mRNA-positive cells and grains per cell were quantified with the help of a computer-assisted image analysis system. Enkephalin (ENK)-mRNA levels were 128% higher in ADX + CORT ($p<0.001$) and 81% higher in ADX + DOCA ($p<0.01$) compared to 3 day sham treated rats. There were no significant differences in tachykinin-mRNA levels after 3 days or ENK-mRNA levels after 7 days. From these results we conclude that CORT and DOCA have similar effects on striatal ENK-mRNA levels. Whether CORT and DOCA bind to their cognate receptors or whether glucocorticoid receptors are occupied by DOCA in ADXed animals to effect increases in striatal ENK-mRNA levels remains to be determined.

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733.2

PROLACTIN (PRL) INCREASES TYROSINE HYDROXYLASE (TH) ACTIVITY IN FETAL HYPOTHALAMIC CELLS. L. A. Arbogast* and J. L. Voogt, Dept. of Physiology, University of Kansas Medical Center, Kansas City, KS 66160-7401

A short loop feedback mechanism provides the main control of PRL secretion *in vivo*. The tuberoinfundibular dopaminergic neurons are a site for PRL feedback, such that high circulating PRL levels increase the synthesis and secretion of dopamine, the major PRL inhibiting hormone. TH is the rate-limiting enzyme in the dopaminergic biosynthetic pathway and thus a key control point. However, studies on intracellular mechanisms for PRL's action have been hampered by the lack of an *in vitro* model. The aims of this study were: 1) to evaluate media composition on the ability of PRL to increase TH activity *in vitro* and 2) to establish time and concentration dependency for PRL action on TH activity. Ventral hypothalamic cells of fetal day 19-20 rats were dispersed with trypsin and plated at 200,000 cells/well and maintained for 10 days. TH activity was determined by incubating cells for 1 hour with 100 μM brocresine, a dihydroxyphenylalanine (DOPA) decarboxylase inhibitor, and measuring DOPA accumulation in the medium. Exp 1: Hypothalamic cells were incubated for 10 days in either a serum-free defined or a serum (5% fetal bovine serum)-containing medium with either low (5 mM) or high (25 mM) potassium (K^+) concentrations. Rat PRL was included in some wells for the 10 days *in vitro*. PRL did not alter TH activity in serum-free medium with either low or high K^+ or in serum-containing medium with low K^+. However, TH activity was significantly increased to 162%, 177% or 172% of control values after 10 days of rPRL-treatment (10, 100 or 1000 ng/ml, respectively). Exp 2: Hypothalamic cells were treated with rPRL (100 ng/ml) for various times. TH activity was not altered by 1, 6 or 12 hours of PRL-treatment, but was increased to 140-145% of control values after 1-3 days, and further increased to 180-185% after 5-10 days. Conclusions: 1) A factor present in fetal bovine serum imparts PRL responsiveness to hypothalamic dopamine neurons *in vitro*. 2) The 24 hour delay for TH responsiveness to PRL to be expressed *in vitro* is similar to the time course *in vivo*. 3) The effective PRL concentrations are within the physiological range of circulating PRL levels *in vivo*. Supported by NIH grant HD24190.

733.3

MU OPIOID RECEPTOR-LIKE IMMUNOREACTIVITY IN THE RAT MEDIAN EMINENCE : DISTRIBUTION AND PLASTICITY IN EXPRESSION. P. Ciofi*[a], F. Vandenbulcke[a], S. Dutoit[a], D. Deneux[a], A. Mansour[b], S.J. Watson[b], G. Tramu[c] and J.C. Beauvillain[a]. [a]U422 INSERM, Place de Verdun, 59045 Lille (France); [b]Mental Health Res. Inst., Univ. Michigan, Ann Arbor, MI 48109; [c]Lab. Neurocyto. Fonct., URA CNRS 339, Univ. Bordeaux I, 33405 Talence (France).

Opioid peptides (OP) are thought to regulate adenohypophysial functions indirectly through hypothalamic circuitry rather than by a direct action at the gland itself. The presence of OP in neuroendocrine terminals (NT) of the median eminence (ME) suggests OP modulate the neurohumoral output via autocrine/paracrine mechanisms. In line with this, we studied the distribution of the mu-receptor in the ME of the adult male rat using an affinity purified antiserum directed at the C-t of the recently cloned rat sequence (Mol. Pharmacol. 44:8; Neuron 11:903; PNAS 90:10230).

Immunocytochemical single or double staining techniques at the light microscopic (fluorescence) or ultrastructural (DAB/peroxidase-colloidal gold combination) levels allowed us to ascribe the ME mu-immunoreactivity (ir) to the gonadoliberin (GnRH)ergic endings, the corticoliberin (CRF)ergic endings and the tanycytes. At the ultrastructural level, after DAB/peroxidase pre-embbeding staining, mu-ir in NT was observed : in dense core vesicles, in the form of sub-membrane patches or in the form of restricted as well as extended thickenings of the membrane, with no strict polarity respectively to the pericapillary space. Mu-ir NT were seen apposed to other NT or to tanycytic processes. In the tanycytes, mu-ir was observed in the form of intracytoplasmic or sub-membrane patches as well as invading spine-like processes.

Orchidectomy and adrenalectomy each resulted in a marked decrease in mu-ir of GnRH and CRF endings, respectively.

These results (1) strenghten the concept of opiatergic autocriny and paracriny in the ME : the GnRH axis do not contain OP while the CRF axis contain enkephalins, and (2) have to be paralleled with the known inhibitory and stimulatory effects of OP on GnRH and CRF axes, respectively.

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733.4

CHOLECYSTOKININ (CCK) INDUCES cFOS EXPRESSION IN BRAINSTEM CATECHOLAMINE CONTAINING NEURONS OF THE RHESUS MONKEY DA Schreihofer*, L Rinaman, JL Cameron, GE Hoffman, JG Verbalis Depts of Neuroscience, Psychiatry, Cell Biology and Physiology, Neurobiology, and Medicine, University of Pittsburgh, Pittsburgh, PA 15260

In monkeys and other species, systemically administered CCK stimulates the release of many pituitary hormones. In rats, the release of oxytocin and ACTH appear to be mediated by CCK-induced activation of vagal afferents that, in turn, activate medullary projections from the nucleus tractus solatarius (NTS) and ventrolateral medulla (VLM) to the hypothalamus. The current study examined the hypothesis that CCK activates similar ascending pathways in adult male rhesus monkeys. Monkeys were given CCK (3 or 15μg/kg iv; n=3 and 2, respectively) or saline vehicle (n=2). Two additional bilaterally vagotomized animals were treated with 15μg/kg CCK. Seventy five minutes after the infusion of CCK, monkeys were deeply anesthetized and perfused with 4% paraformaldehyde. Every sixth section (30μm) through the NTS was processed for immunocytochemical detection of the immediate early gene product cFos as a marker of neuronal activation. Because CCK activates catecholamine neurons that project to the hypothalamus in rats, sections were also stained for tyrosine hydroxylase (TH). Very few cFos positive neurons were present in tissue sections from control animals. In contrast, numerous cFos positive neurons were visualized in the NTS and VLM of monkeys treated with both doses of CCK, and many of the activated neurons were catecholaminergic (TH positive). Vagotomy virtually eliminated CCK-induced cFos expression in the NTS and VLM. These results indicate that CCK activates medullary neurons in the monkey, including many catecholaminergic neurons in the NTS and VLM, and that this activation depends on vagal afferents. These results are consistent with previous studies in rats and suggest common mechanisms of CCK action across these species.

733.5

SEXUAL DIFFERENCES IN THE CIRCADIAN CHANGES OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURON ACTIVITY IN THE RAT: THE ROLE OF THE CHOLINERGIC CONTROL. K.R. Shieh, S.H.H. Chan and J.T. Pan, Inst. Physiol., Natl. Yang-Ming Univ., Taipei, Taiwan R.O.C.

We recently reported that a circadian change of the tuberoinfundibular dopaminergic (TIDA) neuron activity exists in ovariectomized rats treated with or without estrogen (1), and the endogenous cholinergic system is responsible for its induction (2). Whether a similar rhythm exists in intact female or male rats was the focus of this study. In intact female Sprague-Dawley rats, rhythmic changes in TIDA neuron activity were observed during all stages of the estrous cycle, i.e., proestrus, estrus or diestrus I, and they were nearly identical. No such rhythm, however, was observed in intact or castrated male rats treated with or without estrogen. Central administration of mecamylamine (1 μg/3 μl/rat, icv), a nicotinic receptor antagonist, had no effect on the TIDA neuron activity in intact male rats, no matter it was given in the morning or in the afternoon. It appears that the circadian rhythm of TIDA neuron activity is sexual dimorphic, and may under a differential control by the cholinergic system.

1. Mai LM, Shieh KR, Pan JT. Neuroendocrinology 60:520-6, 1994.
2. Shieh KR, Pan JT. Endocrinology 136(6):xx-xx, 1995. (in press)

733.6

VASOACTIVE INTESTINAL PEPTIDE ANTAGONIST ATTENUATES THE NOCTURNAL PROLACTIN SURGE OF PREGNANCY. K. M. Humpherys, L. A. Arbogast, J. D. Radel* and J. L. Voogt, Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66160-7401.

Nocturnal and diurnal prolactin (PRL) surges occur during the first half of pregnancy in the rat. The PRL surges are circadian in nature and the suprachiasmatic nucleus (SCN) serves as a neural locus for generating the surges. Vasoactive intestinal peptide (VIP) is found in the SCN and has been implicated in the control of PRL release. However, the role of VIP in generating the nocturnal PRL surges during pregnancy is unclear. The aims of this study were: 1) to compare the effects of two different VIP antagonists on the nocturnal PRL surge and 2) to assess the dose response relationship of the more potent antagonist. Exp. 1: Female rats received jugular vein cannulas on day six of pregnancy. On day seven, the experimental rats were infused continuously with [p-Chloro-D-Phe^6, Leu^{17}]-VIP antagonist (0.1 μg/kg-min) between 2200 h and 0700 h. Control rats were infused with saline. Serial blood samples were obtained immediately before infusion and hourly thereafter. Circulating PRL levels during the nocturnal surge in control and VIP antagonist treated pregnant rats were similar at all times examined. Exp. 2: A neurotensin/VIP hybrid, which is a more potent VIP antagonist without VIP agonist activity, was examined at three different doses (0.001, 0.01, and 0.1 μg/kg-min) repeating the protocol described above. In control rats serum PRL levels were low (1-10 ng/ml) between 2200 h and 0100 h, were elevated to 200-300 ng/ml during the peak of the nocturnal PRL surge at 0200 h to 0400 h, and then declined between 0500 h and 0700 h. The groups with the antagonist doses of 0.001 and 0.01 μg/kg-min had plasma PRL levels similar to the control group. In contrast, circulating PRL levels were reduced in the group receiving the dose of 0.1 μg/kg-min by 83%, 74%, 50% and 43% at 0100, 0200, 0300, and 0400 h, respectively. In conclusion, the more potent VIP antagonist attenuated the nocturnal PRL surge of pregnancy suggesting that VIP plays a positive role in generating the surge. Supported by NIH Grant HD24190.

733.7

FOS EXPRESSION IN THE NTS AFTER HYPERTENSIVE STIMULI BUT NOT CCK INJECTIONS CORRESPONDS TO AREAS RETROGRADELY LABELED FROM THE VLM. R.G. Mayne*[1], S.L. Bealer[2], W.R. Crowley[3] and W.E. Armstrong[1]. Depts. of [1]Anat.& Neurobiol., [2]Physiol. & Biophys., and [3]Pharmacol.; Univ. Tenn., Memphis, TN 38163.

Most visceral inputs make their first synapses within the nucleus tractus solitarii (NTS) where information is then relayed to other brain regions that regulate autonomic and neuroendocrine responses. Hypertension and gastric stimuli elicit Fos expression in functionally distinct regions of the NTS and in overlapping regions of the ventrolateral medulla (VLM) of rats. Fos-like immunoreactivity (FLI) and retrograde tracing were used to determine if the NTS neurons that respond to phenylephrine-induced hypertension or stimulation from cholecystokinin (CCK) project to the VLM.

Male rats were injected with either Rhodamine-labeled beads or Fluoro-Gold unilaterally into the VLM and allowed to survive one week. Then these rats were either made hypertensive (30-40 mm Hg over baseline) by constant intravenous infusion of phenylephrine (5-10 µg/kg/min for 90 mins) or received an injection of CCK (100 µg/kg, i.p.). Tissue sections were then processed for FLI.

Retrogradely labeled somata were distributed in the NTS similar to hypertension-induced FLI but were distinct from neurons expressing Fos after CCK injection. Hypertensive stimuli elicited FLI in the dorsal part of the commissural NTS caudal to area postrema, overlapping the distribution of retrogradely labeled neurons. In contrast, after CCK injection FLI was scattered throughout the commissural NTS caudal to the area postrema. At the level of the area postrema, the majority of Fos-labeled neurons after hypertension were in the dorsolateral and medial subnuclei. Retrograde labeling was present in the dorsolateral and sparsely in the medial subnuclei, but not in the commissural NTS at this level. After CCK, FLI was present primarily in the commissural nucleus, the medial subnucleus, and area postrema at this level. These results show that NTS neurons responding to hypertension but not CCK overlap with a population of neurons that project to the VLM. Supported by NIH grants NS 07323 (RGM), NS 23941 (WEA), HL 25877 (SLB) and HD 20074 (WRC).

733.8

NADPH-DIAPHORASE ACTIVITY AND FOS EXPRESSION IN BRAIN NUCLEI FOLLOWING NITROGLYCERIN ADMINISTRATION. Cristina Tassorelli* and Shirley A. Joseph. Department of Surgery, Division of Neurological Surgery, University of Rochester Medical Center, Rochester, NY 14642 and *Department of Neurology "C. Mondino" Foundation, University of Pavia, I 27100.

Organic nitrates are considered nitric oxide (NO) donors since they have been shown to form NO *in vitro* and *in vivo*. Nitroglycerin (NTG) is an organic nitrate which possesses several centrally-mediated effects, some of which have been shown to be influenced by the noradrenergic system. Recently it has been demonstrated that NTG can directly influence the electrical activity of neurons in the nucleus tractus solitarius, probably by way of the local release of NO. In a previous report we have shown that systemic NTG is able to induce Fos expression in brain nuclei which are known to contain also the NO synthesizing enzyme (NOS). Neuronal NADPH-diaphorase (NADPH-d) has been shown to be a NOS. In this study NADPH-d histochemistry was used to evaluate the distribution of the neurons which express Fos following the systemic administration of NTG within the pool of neurons which contain the NOS.

These data demonstrate that Fos was significantly co-localized with NADPH-d activity in specific subsets of neurons in the paraventricular and supraoptic nuclei of the hypothalamus. Only a few neurons were double labeled for Fos and NADPH-d activity in the brainstem, but NADPH-d positive fibers were found consistently co-distributed with Fos immunoreactive neurons in the locus coeruleus, parabrachial nucleus, nucleus tractus solitarius and spinal trigeminal nucleus caudalis.

These findings demonstrate that NTG administration activates a selective group of neurons which are a source of NO or which are in close proximity with neuronal processes containing NOS and suggest a possible involvement of NO as a mediator of the central effect of NTG. Supported by grants NS21323 (S.A.J.) and AI94.00462.04 (C.T.)

733.9

ALPHA-2 ADRENOCEPTORS MODULATE HYPOTHALAMIC FOS-LIKE IMMUNOREACTIVITY IN THE CONSCIOUS SUCKLED RAT.

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The supraoptic nucleus (SON) has a high density of alpha-2 adrenoceptors and previous studies have indicated a role for these receptors in controlling the activity of SON neurones in the anaesthetised suckled rat. To investigate the role of alpha-2 adrenoceptor mediated transmission in the unanaesthetised animal we have examined the effects of a systemically administered alpha-2 adrenoceptor agonist, clonidine and an antagonist, idazoxan, on SON Fos-like immunoreactivity (Fos-LI) in the conscious suckled rat. Lactating Wistar rats, chronically implanted with an intravenous catheter, were separated from their litter for two hours which was then weighed. The mothers were then given an intravenous injection of either clonidine (50µg/kg, n=7), idazoxan (0.5mg/kg, n=6) or saline (0.2ml, n=7). The pups were returned and maternal behaviour recorded for 90 minutes then the litters were reweighed. Rats were then killed and brain sections immunocytochemically processed for Fos-LI. Saline-treated animals had a mean litter weight gain of 7.9±1.4g and displayed 7.9±0.8 milk-ejections in 90 minute. Fos-LI was observed in the SON of all saline-treated rats (99.9±4.9 cells/section). Clonidine reduced both litter weight gain (5.8±1.4g) and milk-ejection number (5.1±1.1) and SON Fos-LI was also significantly reduced compared to saline-treated controls (19.4±2.7 cells/section, P<0.01 Mann Whitney U-test). Idazoxan also decreased litter weight gain (4.2±0.5g) and milk-ejection number (4.0±1.4) in addition to reducing Fos-LI in the SON (30.9±2.8 cells/section, P<0.05 Mann Whitney U-test). These results indicate that activation of neurones in the SON can be attenuated by systemic injection of clonidine and idazoxan providing evidence for the involvement of alpha-2 adrenoceptors in the control of SON neuronal activity in the conscious lactating rat.

733.10

EFFECTS OF ACUTE AND CHRONIC HALOPERIDOL TREATMENT ON MELATONIN SYNTHESIS IN RAT PINEAL GLAND. L. Steardo*, P. Monteleone+, M. Persichella, M. Mazzoccoli, A. Attanasio and V. Cuomo. Dept. of Pharmacology, Universitary Medical School, Bari (Italy). +Dept. of Psychiatry, Universitary Medical School, Naples (Italy).

The nocturnal production of melatonin by the mammalian pineal gland occurs in response to noradrenergic stimulation. It is well established that noradrenaline (NA) acting on a β_1 receptor, and to a lesser extent on a α_1 receptor, activates the rate-limiting enzyme serotonin-N-acetyltransferase (NAT). However, recent data indicate that receptors others than NA can play an important modulatory role in the process of melatonin production. Preliminary evidence from in vitro studies has been provided suggesting that dopamine (DA) may affect melatonin synthesis in rats. To characterize the precise role of this cathecolamine on the physiological activity of the rat pineal gland, the authors have investigated the effects of acute and chronic administration of haloperidol (1mg/kg, i.p.) on the nocturnal NAT activity and melatonin pineal content.

Results have shown that, as compared to saline, both acute and chronic haloperidol treatment significantly antagonized the nocturnal increase of pineal NAT activity ($p < 0.0005$ for the acute experiment, and $p < 0.001$ for the chronic one) and melatonin levels ($p < 0.001$ for both acute and chronic experiments).

Therefore, the present data support the view that DA partecipates to the complex mechanisms which regulate the physiological production of melatonin in the rat pineal gland.

733.11

VAGINOCERVICAL STIMULATION INDUCES C-FOS

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It has been published that vagino-cervical stimulation (VCS), induces reproductive behaviors in female animals. In the present study, we investigated the effect of VCS to induce Fos in different brain nuclei in the female albino rat. For one week, the animals were familiarized with the investigator, then the animals were placed in a special box and a Foley catheter was inserted into the vagina until the tip reached the cervix. 0.5-0.8 ml of room temperature water was infused into the balloon and it remained distended for 5-20 min. In the control group, the catheter was inserted but not distended for the same period of time. The animals were allowed to survive for a 60 min after VCS was completed. Then, the animals were deeply anaesthetized and perfused with 0.9% NaCl solution followed by 500 ml of 4% paraformaldehyde. Fos immunoreactivity in the nuclei of neurons (Fos labelled cells) was visualized by conventional ABC immunohistochemistry using DAB dehydrochloride as the chromogen agent. Fos-like immunoreactivity was induced differentially depending upon the duration of the VCS. The nuclei mainly labelled were: lateral septal, mPOA, diagonal band of Broca, BNST, VMH, medial amygdala, paratenial thalamus, paraventricular thalamus, lateral central gray. Control animals showed the same labelling pattern as the animals which received VCS for 5-10 min. On the contrary the group of 15 min up of VCS shown a higher amount of labell cells. In contrast, animals which received greater than 15 min of VCS had more labelled cells. The animal that in diestrus-proestrus had more labelled cells than those in diestrus. These results have important implications concerning the neural mechanisms involved in the control of female sexual behavior.

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733.12

DOPAMINERGIC ANTAGONISTS ENHANCED THE NEUROSTEROID-INDUCED MODULATION OF THE NMDA-EVOKED [^{3}H]NE OVERFLOW IN THE RAT HIPPOCAMPUS. F. Villemain*, F.P. Monnet & E.E. Baulieu, DRC-DPIM AP-HP, CNRS-UPR2212 & INSERM-U33, F-94276 Kremlin Bicêtre, France.

We have recently shown that both dehydroepiandrosterone sulfate (DHEAS) and pregnenolone sulfate (PREGS) potentiated and reduced, respectively, concentration-dependently the *N*-methyl-D-aspartate(NMDA)-evoked [^{3}H]norepinephrine ([^{3}H]NE) overflow from preloaded hippocampal slices of spayed rats. While haloperidol, via acting on sigma sites antagonized both DHEAS- and PREGS-induced modulations of the NMDA response, spiperone, another butyrophenone with low affinity for sigma sites enhanced the neurosteroid-mediated responses. Spiperone, like haloperidol, binds to dopaminergic, serotoninergic and cholinergic sites. The present experiments were thus carried out to determine the nature of the effect of spiperone on neurosteroid-induced modulation of the NMDA response in the *in vitro* release model by measuring actions of various (non)selective ligands for these sites. Hippocampal slices from spayed Sprague-Dawley rats were incubated with 0.1 µmol/l [^{3}H]NE for 30 min and superfused continuously with Mg^{++}-free Krebs'solution containing PREGS or DHEAS in the presence or absence of the following antipsychotics: haloperidol, chlorpromazine, sulpiride as well as ritanserine and atropine. 40 min later, the [^{3}H]NE overflow was evoked by NMDA (200 µM). In the presence of the D_2 dopaminergic antagonists sulpiride and chlorpromazine, PREGS (300 nM) failed to reduce, whereas DHEAS (300 nM) further potentiated the NMDA-evoked [^{3}H]NE overflow. Conversely, ritanserine and atropine, devoid of affinity for dopaminergic sites, were ineffective in modifying both DHEAS- and PREGS-induced modulation of the NMDA response. The present results constitute the first evidence supporting the notion that neurosteroids, such as DHEAS and PREGS, interact with central monoaminergic neurotransmission and support the notion that neurosteroids may constitute relevant probes for neuropsychiatric diseases.

733.13

SERUM PREVENTS STELLATION OF CULTURED PITUICYTES INDUCED BY CYCLIC NUCLEOTIDE. K.D. Ramsell* and P. Cobbett. Dept. Pharmacol./Toxicol. and Neuroscience Program, Michigan State Univ., E. Lansing, MI 48824.

Pituicytes (neurohypophysial astroglia) from adult rats exhibit morphological changes when intracellular cAMP is raised by activation of adenylate cyclase (Ramsell & Cobbett, Soc. Neurosci. Abstr.:**20**,96,1994). Since a component of serum, lysophosphatidic acid, reverses β-adrenoreceptor mediated stellation of rat C6 glioma cells (Koschel & Tas, Exp. Cell Res.: **206**,162-166,1993), we have investigated the effects of newborn calf serum (NCS) on cAMP mediated stellation of cultured pituicytes. The fraction of stellate pituicytes was significantly increased when cultures were incubated in medium containing forskolin (5μM, 0.94±0.01) compared to that seen in control medium (0.06±0.01). The effect of forskolin was significantly reduced when 0.5% NCS was also included in the incubation medium. NCS also blocked stellation induced by the phosphodiesterase inhibitor IBMX (50μM) and by 8bromo-cAMP (100μM). These data indicate that serum prevents stellation of pituicytes by a mechanism independent of intracellular cAMP concentration. Supported by NINDS (NS28206).

733.14

CHARACTERIZATION AND EXPRESSION OF LOBSTER PREPROHORMONES INVOLVED IN METABOLISM, MOLTING AND REPRODUCTION Dominique P.V. De Kleijn[1,3], Susan L. Waddy[2], Gerard J.M. Martens[1], Ronald Verwer[3]* and François Van Herp[1]. 1 Department of Animal Physiology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands. 2 Invertebrate Fisheries Section, Biological Station, St Andrews, New Brunswick, Canada E0G 2X0. 3 Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam

The crustacean hyperglycemic hormones (CHH-A and -B) are primarily involved in the regulation of carbohydrate metabolism while the gonad-inhibiting hormone (GIH), inhibits vitellogenesis and belong to a new neuropeptide family. In order to get more information on the synthesis, storage, release and possible function(s) of CHH-A, CHH-B and GIH during the reproductive cycle, we measured the levels of their mRNAs in the X-organ, their peptide storage in the neurohemal organ and their hemolymph peptide levels at different stages of the female reproductive cycle. For CHH, a high CHH-A mRNA level was found at the previtellogenic stage while CHH-B mRNA levels were higher in the mature as well as in the previtellogenesis stages when compared to the other stages. During previtellogenesis, high storage levels for both CHHs were found in the sinus gland. In the hemolymph, the total amount of CHH (CHH-A plus -B) was high only during maturation. For GIH, a low level of mRNA in the X-Organ and a low amount of the GIH-I isoform in the sinus gland were found only in the immature stage. In contrast, GIH hemolymph levels were high during the immature and previtellogenic stages. Together, we conclude that CHH-A and -B are probably involved in triggering the onset of vitellogenesis and especially CHH-B seems to be responsible for stimulating oocyte maturation before spawning, while GIH prevents the start of vitellogenesis in the ovary. Moreover, the balance between the hemolymph levels of CHHs and GIH may regulate the synchronization of reproduction and molting during the bi-annual reproductive cycle of the American lobster.

733.15

CALCIUM ENTRY FROM EXTRACELLULAR FLUID IS NOT REQUIRED FOR PROLONGED HORMONE SECRETION FROM *APLYSIA* NEUROENDOCRINE CELLS. N. L. Wayne*, Dept. Physiology, UCLA School of Medicine, Los Angeles, CA 90024.

In most neurosecretory systems investigated, membrane depolarization opens voltage-gated calcium channels and Ca^{++} enters the cell from extracellular fluid, stimulating exocytosis of secretory granule contents. This does not seem to be the case for neuroendocrine bag cells of *Aplysia* that secrete the peptide hormone egg-laying hormone (ELH). Recently we have shown that once a bag-cell electrical afterdischarge is initiated, Ca^{++} influx is not required for prolonged ELH secretion (Wayne and Frumovitz, 1995). However, it is possible that brief Ca^{++} entry during the afterdischarge triggers prolonged Ca^{++} release from intracellular stores, thereby stimulating prolonged peptide secretion. In an initial test of this hypothesis, bag-cell preparations maintained *in vitro* were treated with the calcium ionophore X537A (50 μM) dissolved in artificial seawater (ASW) containing 5 mM Ca^{++}/10 mM EGTA. This ionophore can carry Ca^{++} from extracellular and intracellular sources. By blocking entry of Ca^{++} from extracellular fluid with the calcium chelator, the only source of Ca^{++} should be from intracellular stores. This treatment stimulated significant ELH release during the entire 120-min treatment period in the absence of action-potential firing. Total ELH release from preparations treated with X537A (1.2 ± 0.6 μg/ml, n=3) was similar to that from a control group treated with normal ASW and in which secretion was stimulated by an electrical afterdischarge (1.5 ± 0.3 μg/ml, n=5). These results show that a sustained rise in intracellular calcium is sufficient to stimulate ELH release in the absence of an electrical afterdischarge, and are consistent with a model of secretion in which peptide hormone release does not require Ca^{++} influx from extracellular fluid. Supported by NIH NS-33548.

733.16

PATTERNS OF AFTERDISCHARGE (AD) AND EGG-LAYING HORMONE (ELH) RELEASE FROM BAG CELLS UNDER DAILY ELECTRICAL STIMULATION. W. Lee* and N. L. Wayne. Dept. of Physiology, UCLA Sch. of Med., Los Angeles, CA 90024.

ELH of the marine mollusk, *Aplysia*, is synthesized by neuroendocrine bag cells and its secretion is triggered by an electrical AD. During the breeding season, ELH synthesis rate is elevated, and egg-laying behavior often occurs daily in response to ELH secretion. To investigate how ELH synthesis and the pattern of its secretion are regulated when the demand is high, we used a bag cell preparation that remains stable for days *in vitro* (n=4). To determine their electrophysiological and ELH secretory capacities with repeated stimulation, bag cells were dissected from the abdominal ganglion, and individual clusters were separated and placed in artificial sea water containing 100 μCi/ml ^{3}H-leucine for 20-24 hours, and then electrically stimulated on each successive day for up to 5 days, which would mimic daily bouts of AD and ELH release that induce egg laying in freely-behaving animals. During these days each cluster was maintained in HEPES-buffered Eagle's medium II except during the 130-min experimental period. The results showed that while the pattern of action potential firing remained stable, total ELH release per day gradually decreased over time. This gradual decline in release was shown to be a function of repeated stimulation rather than time in culture. Also, secretory patterns of ^{3}H-labeled acid-precipitable peptides appeared to be consistent with those of ELH, but this release of newly synthesized peptides was highest on the third day of the experiment. These results suggest that daily release of ELH (as often occurs during breeding season) can eventually exhaust the synthetic/secretory machinery underlying peptide secretion. Supported by NIH-NS33548 (NLW).

733.17

LONG DISTANCE DIFFUSION OF DEXTRAN ALONG NERVE FIBRE BUNDLES. ASPECTS ON VOLUME TRANSMISSION

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The mapping out of preferential diffusion pathways in the living brain is basic to the concept of Volume Transmission. Texas Red labelled dextran, a marker for the extracellular space, was injected from the dorsal side of the brain unilaterally into the neostriatum in rats (0.3 - 30 μg/μl) and evaluated 1 min to 5 h later. Confocal laser microscopy including co-localization analysis in combination with myelin basic protein staining demonstrated that the Texas Red labelled dextran (5 min, 3 μg/μl) was associated with small channel-like pathways parallel to the myelinated fiber bundles and separate from the MBP-1 immunoreactivity of the fiber bundles. In addition, strong labelling along the myelinated fibre bundles was seen reaching into the entire neostriatum as well as into the corpus callosum and external capsule on the contralateral side. A diffusion in the neuropil was observed with a clearance starting after 30 min. A marked cellular uptake and accumulation of labelled dextran was found in putative perivascular pericytes. Thus, in the living brain preferential extracellular fluid pathways for diffusion may exist along myelinated nerve fiber bundles.

734.1

MAPPING MICTURITION CONTROL AREAS IN THE CENTRAL NERVOUS SYSTEM WITH POSITRON EMISSION TOMOGRAPHY (PET)

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Little is known about the brain structures controlling micturition or voiding. Indirect evidence from patients with cerebrovascular lesions, large brain tumors or lobotomies suggest that voluntary control of micturition in humans depends on the integrity of the medial surface of the frontal lobe (including the anterior cingulate gyrus) and the septal and preoptic regions of the hypothalamus. In this study an attempt was made to identify cortical and subcortical areas involved in micturition in healthy human volunteers.

Cerebral activation was monitored in 7 right-handed male volunteers (23-50 years) using a CTI ECAT 951/31 whole body positron emission tomograph. Changes in regional cerebral blood flow (rCBF) were measured using the intravenous radioactively labeled water ($H_2{}^{15}O$) bolus technique. An injection of 1.85 MBq $H_2{}^{15}O$ was given for each run. Scanning was made during 3 successive conditions: 1) with a filled bladder, 2) during micturition, and 3) with an empty bladder. Shifting from the condition with a filled bladder to the condition in which the micturition took place indeed altered the pattern of rCBF. During micturition, a significant increase ($p<0.01$) in rCBF was found in the ventral hypothalamus, parts of the striatum, the left anterior cingulate gyrus, the caudal periaqueductal gray (PAG), and the possible human M-region or pontine micturition center. The results support previous animal research which indicated that the M-region, the PAG and the preoptic area of the hypothalamus play a central role in the control of micturition (Holstege, 1987; Blok and Holstege, 1994). The results suggest a remarkable similarity between the organization of micturition in cats and humans. Understanding the control of micturition in the human might help to understand the pathophysiology of urge incontinence, one of the major problems in elderly.

734.2

BRAIN AND SPINAL NEURONS IDENTIFIED IN THE FEMALE RAT AFTER INJECTION OF PSEUDORABIES VIRUS (PRV) INTO THE BLADDER BODY, BASE AND EXTERNAL URETHRAL SPHINCTER (EUS). L. Marson,* Division of Urology, University of North Carolina School of Medicine, Chapel Hill, NC 27599

Bladder disorders are common in females. Continence and voiding are dependent upon the functional integration and co-ordination of detrusor and urethral mechanisms. These mechanisms are modulated by neural inputs from the brain and spinal cord. Transneuronal tracing with PRV was used to identify the spinal and brain neurons that innervate the female bladder and urethral sphincter. Bartha's K strain of PRV was injected into either the bladder body, bladder base or EUS (1-4 µl. 10^8pfu/ml). Rats were perfused 4 or 5 days after the injections. Bladder injections labelled neurons in the IML and dorsal grey commissure (DGC) of segments L6-S1 and T13-L3 and in the medial cord of the intervening segments. After a 5 day survival, PRV cells were also found in laminae II and III of the dorsal horn. Fewer and more localized cells were found after PRV injections of the bladder base. PRV injected into the EUS, labelled motoneurons in the dorsolateral subdivision of Onuf's nucleus. Longer survival times also labelled neurons in the dorsomedial and ventrolateral subdivisions of Onuf's nucleus. PRV labelled cells were also found in the IML and DGC of segments L6-S1 and T13-L3, after EUS injections. In the brain, areas common to both bladder and EUS injections were: paragigantocellular and gigantocellular medullary reticular formation, raphe magnus, A5, locus coeruleus and subcoeruleus, Barrington's nucleus and the lateral and ventral central grey. More rostrally PRV cells were found in the posterior, dorsal and lateral hypothalamic areas, tuber cinereum, paraventricular nucleus of the hypothalamus and the preoptic region. These studies demonstrate a high degree of overlap between bladder smooth muscle and EUS striated muscle central nervous system projections. However, some differences were observed in the extent of labelling. (Supported by NIH grants NS29420 and DK49503).

734.3

TRANSYNAPTIC LABELING OF NEURONS WITHIN THE CNS WHICH CONTROL THE BLADDER OR PENIS OF THE CAT. V.Erickson, J.R.Roppolo, A.M.Booth*, M.A.Vizzard, J.P.Card, and W.C.de Groat Depts. of Pharmacol. & Neurosci., Univ. of Pittsburgh, Pgh, PA 15261

This study used the transynaptic tracer pseudorabies virus (PRV) to determine the location of efferent neurons and interneurons within CNS pathways that control the bladder or penis. Under halothane anesthesia PRV (10-20µl, $1.7x10^8$ pfu/ml, Becker strain) was injected unilaterally into the bladder wall or penis of cats. Animals were perfused 60-115 hours later. PRV injected into the bladder, labeled neurons (L-NEU) in the S_1-S_3 and L_1-L_4 segments of the spinal cord with the majority of the L-NEU in S_2 and L_3. In the sacral cord L-NEU were in the lateral, sacral parasympathetic nucleus (SPN), the dorsal commissure (DC), in the superficial layers of the dorsal horn (DH), and a few L-NEU were also in Onuf's nucleus. At the lumbar level L-NEU were in the intermediolateral cell column (IML), in the DC and in the superficial layer of the DH. In animals allowed to survive >96 hours, brainstem labeling was medial to, and overlapping with locus coeruleus (LC) neurons and extending into the periaqueductal grey and the pontine reticular formation. A few L-NEU were also in the raphe nucleus at the level of the pons. In two separate animals either the S_1-S_3 dorsal roots (DRX) or ventral roots (VRX) were transected prior to bladder injection. Following 60 hour survival little labeling was seen the VRX animal. However many labeled preganglionic neurons (PREG) and interneurons were seen in the SPN and DC of the DRX animal. After injection of PRV into the penis L-NEU were in the S_1-S_3 and L_1-L_4 spinal cord with the majority in S_1, rostral S_2 and L_3. In the sacral cord L-NEU were in the DC, the ventral horn and the medial SPN. L-NEU in the lumbar cord were primarily in the IML. L-NEU were also seen in the brainstem medial to the brachium conjunctivum and lateral to the LC. This data suggests: 1) that penile PREG are adjacent to but separate from bladder PREG in the sacral cord, 2) there is considerable overlap of the interneurons serving the two organs and 3) that brainstem neurons controlling bladder or penis are present in separate regions of the pons. [Supported by NINDS N01-NS-2-2374].

734.4

MODULATION OF EXTERNAL URETHRAL SPHINCTER (EUS) BY MICROSTIMULATION OF THE SACRAL SPINAL CORD. J.R.Roppolo*, A.M.Booth & W.C.de Groat. Dept. of Pharmacol., Univ. of Pittsburgh, School of Medicine, Pgh., PA 15261.

The purpose of the present study was to determine sites within the sacral spinal cord where electrical stimulation with fine tipped microelectrodes produced EUS contraction or relaxation, either in the presence or absence of accompanying bladder contractions. Both α-chloralose anesthetized and precollicular decerebrate unanesthetized cats were used in this study. EUS pressure was recorded via a catheter passed transurethrally with its tip located at the level of the EUS. Bladder pressure was recorded isovolumetrically by a catheter through the bladder dome. Sites which produced EUS contractions were in S_2 and caudal S_1 of both anesthetized and decerebrate cats and included: (1) areas on or near (200-400µ deep) the surface of the spinal cord at the dorsal root entry zone (DREZ) and 200-400µ medial or lateral to the DREZ; (2) deep in the ventral horn and ventral funiculus near the cell bodies and axons of pudendal motoneurons; (3) in the lateral funiculus, ventrolateral to the dorsal horn. Although sites which inhibit EUS were sometimes identified in anesthetized animals the EUS relaxations were usually of small (10-15cm H_2O) magnitude and often difficult to reproduce even in the same animal. EUS relaxation was best demonstrated in decerebrate unanesthetized cats. Sites which produce EUS relaxation were often preceded by a small EUS contraction. Sites which produced EUS inhibition include: (1) sites in and near the sacral parasympathetic nucleus (SPN), (2) the dorsal horn just dorsal and dorsomedial to the SPN and (3) along the lateral edge of the ventral horn. Evoked bladder activity was not correlated with the magnitude of the EUS relaxation. Some sites (ie. deep in ventral horn and ventral funiculus of S_2) produced large bladder contractions with little or no EUS inhibition (often small EUS contractions were seen); while small bladder contractions produced by dorsal horn stimulation were often associated with large EUS relaxation. These studies suggest that focal microstimulation of the sacral spinal cord may be a useful technique for producing bladder emptying. [Supported by N01-NS-2-2374].

734.5

PROPERTIES OF NEURONS IN THE REGION OF THE SACRAL PARASYMPATHETIC NUCLEUS (SPN) IN THE ISOLATED SPINAL CORD OF THE NEONATAL RAT. Y.B.Yu*, A.M.Booth, W.C.de Groat. Dept. Pharmacology, Univ. Pittsburgh, Pittsburgh, PA 15261.

Parasympathetic preganglionic neurons (PGN) and interneurons (INT) in the L6-S1 spinal cord are involved in the control of the urogenital tract and distal bowel. The present study examined the properties of these neurons in the isolated spinal cord of neonatal rats (6-11 days old). Extracellular recording in the SPN region identified two types of neurons. PGN were activated antidromically at high frequencies (100 Hz) at latencies of 7.5-20 ms by electrical stimulation (EStim) of ventral roots (VRT). Some neurons responded synaptically to EStim of dorsal roots (DRT). The synaptic responses consisted of single spikes and followed only low frequencies (less than 1 Hz) of EStim. INT did not respond to VRT EStim but did fire synaptically at 10-25 ms latency to DRT EStim. INT exhibited multiple spikes (2-4) in response to a single DRT EStim and followed frequencies up to 2-5 Hz. DRT EStim elicited early (4-13 ms latency) and late reflexes (up to 30 ms latency) on L6 and S1 VRT's. Neurons labelled by extracellular iontophoresis of biocytin in the SPN region were stellate, oval or round in shape and ranged from 10 to 20 µm in diameter. Some neurons presumably PGN which exhibited long axon-like processes extending deep into the ventral horn had dendrites which extended into the lateral funiculus and medially toward the central canal. Small round or oval neurons in the SPN had dorsoventrally oriented processes. Neurons with long mediolateral dendrites were identified in the region of the dorsal commissure. These neurons might be labelled by retrograde axonal transport of biocytin from the SPN. It is concluded that some PGN and INT in the SPN are activated by primary afferent inputs via polysynaptic pathways. Thus the late component (25-30 ms) of the DRT to VRT reflexes could reflect in part autonomic neuron firing.

734.6

C-*FOS* EXPRESSION IN SPINAL NEURONS AFTER IRRITATION OF THE LOWER URINARY TRACT DEPENDS ON SYNERGISTIC INTERACTIONS BETWEEN NMDA AND AMPA GLUTAMATERGIC TRANSMISSION. H. Kakizaki, M. Yoshiyama and W.C. de Groat*. Dept. of Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA 15261

Chemical irritation of the lower urinary tract (LUT) of the rat increases the expression of immediate early gene c-*fos* in the dorsal horn (DH), dorsal commissure (DCM) and intermediolateral region including parasympathetic nucleus (SPN) of the L6-S1 spinal cord. This c-*fos* expression is decreased by a large dose (3.5 mg/kg i.v.) of NMDA receptor antagonist MK-801 (Birder & de Groat, 1992). The role of glutamatergic synapses in c-*fos* expression following LUT irritation was further examined using a selective, competitive AMPA receptor antagonist (LY 215490). Chemical irritation was performed by a continuous transvesical infusion of 1% acetic acid in urethane anesthetized female Wistar rats. The total number of c-*fos*-positive cells following 2-hr LUT irritation was 110±2.4 cells/L6 section (45±1.3, 34±1.0 and 31±1.1 cells/L6 section in DCM, SPN and DH, respectively). LY 215490, administered 1 hr before LUT irritation, decreased significantly the number of c-*fos*-positive cells in DCM and SPN in a dose-dependent manner (mean % decrease following 3 and 10 mg/kg i.v. : 22% and 51% in DCM, 25% and 43% in SPN, respectively). In DH, only a large dose of LY 215490 (10 mg/kg, i.v.) decreased (27-33%) the number of c-*fos*-positive cells. A low dose of either MK-801 (15 min before irritation) or LY 215490 alone (1 mg/kg i.v. of each) did not alter c-*fos* expression. However, combination of the low doses of MK-801 and LY 215490 significantly decreased the number of c-*fos*-positive cells in all regions of the spinal cord (mean % decrease: 29 - 44%). These results indicate that AMPA as well as NMDA receptors are involved in the spinal processing of nociceptive input from the LUT and that these two glutamatergic receptors play a synergistic role in visceral nociceptive processing.

734.7

DENERVTION AND INFLAMMATION INDUCED INCREASE OF LOW -AFFINITY NGF RECEPTOR IMMUNOREACTIVITY IN THE RAT URINARY BLADDER. Y. Wakabayashi, A. Buchan* and Y. N. Kwok. Dept. of Physiology, University of British Columbia, Vancouver, B.C. Canada V6T 1Z3

The level of NGF in the urinary bladder has been shown to be increased following urethral obstruction, denervation or inflammation, thus suggesting that NGF may play a role in the control of bladder function. However, little is known about the distribution and localization of NGF receptors in the urinary bladder. The objective of the present experiments was to examine immunohistochemically the distribution and localization of the low-affinity NGF receptor (LNGFR), which binds all neurotrophins, in the male adult rat urinary bladder using a specific antibody (192 IgG). In controls LNGFR positive fiber bundles were shown to be present in the muscle layer. In cyclophosphamide (CYP)-induced cystitis animals, LNGFR positive fine fibers were observed in the muscle layer 2 days after CYP treatment (150 mg/kg, i.p.). Three days after unilateral major pelvic ganglionectomy, LNGFR immunoreactivity was also increased in fine fibers of the muscle layer of the denervated side, and this increase peaked at 7 days. Electron microscopic examination showed that reaction products were located on the surface of Schwann cells and on the interface of axons and Schwann cells. Although the significance of the present findings and the role of LNGFR in the urinary bladder are not certain, these receptors may play a role in nerve regeneration, sprouting and/or neural plasticity. (Supported by BCHRF).

734.8

REGULATION OF MICTURITION BY CORTICOTROPIN-RELEASING HORMONE (CRH) FROM BARRINGTON'S NUCLEUS. L. A. Pavcovich and R. J. Valentino*. Dept. of Psychiatry, Hahnemann Univ., Philadelphia, PA 19102.

Barrington's nucleus, a pontine nucleus implicated in micturition, contains numerous CRH neurons that project to the spinal parasympathetic nucleus that innervates the bladder. This study characterized the effects of selective chemical stimulation of Barrington's nucleus on bladder function, and determined the role of CRH-Barrington's projections in these effects. Bladder pressure was continuously recorded during intracerebral glutamate microinjection (10 mM, 15-60 nl) in halothane-anesthetized rats. Selective chemical activation of Barrington's nucleus by glutamate microinjection evoked bladder contractions corresponding to an increase in pressure of 1-9 mm Hg, and this effect was regionally specific. The CRH antagonist, D-Phe CRH_{12-41}, (3 μg) administered intrathecally (it) increased the magnitude of Barrington's stimulated contractions. Moreover, the magnitude of this increase was correlated with the magnitude of chemical activation, eg., bladder contractions evoked by 15, 30 and 60 nl of glutamate were increased by 34±13%, 87±21% and 110±23%, respectively (n=7-9). In contrast to D-Phe CRH_{12-41}, CRH (3 μg, it) decreased Barrington's stimulated bladder contractions (n=7), and artificial cerebrospinal fluid (3 μl, it; n=5) had no effect. CRH did not alter bladder contractions evoked by it administered N-methyl-D-aspartate (10 mM). The results suggest that activation of Barrington's nucleus releases an excitatory neurotransmitter responsible for bladder contractions, and CRH, which presynaptically inhibits this neurotransmitter. Through this interaction, CRH may regulate the micturition reflex.

734.9

SENSORY INNERVATION OF THE RAT LOWER URINARY TRACT: A ROLE FOR NITRIC OXIDE? K. Persson*, K. Johansson, P. Alm, B. Larsson and K.-E. Andersson. Departments of Clinical Pharmacology, Zoology and Pathology, University of Lund, Lund, Sweden.

Retrograde axonal tracing studies have demonstrated nitric oxide synthase (NOS) positive neuronal structures in segments of the spinal cord which receive afferent input from the bladder and urethra. Thus, besides being an efferent messenger in the lower urinary tract, NO may also be involved in afferent neurotransmission. It is possible that NOS-containing fibres in the detrusor represent sensory fibres, since no clear role of NO in efferent neuromuscular transmission has been established in this tissue. The objective of this study was to perform a morphological investigation of the suburothelial/urothelial innervation of the rat lower urinary tract with special reference to NOS-containing fibres. The bladder and urethra from control and capsaicin treated female Sprague Dawley rats were fixed in 4% formaldehyde and the specimens processed for immunohistochemistry. Calcitonin gene related peptide (CGRP)- and substance P (SP)-immunoreactive (IR) nerves were observed in the suburothelial region of the bladder and urethra. NOS-IR fibres were also observed in the suburothelial region, although more sparse compared to CGRP- and SP-IR fibres. Occasionally, CGRP-, SP- and NOS-IR fibres penetrated into the urothelial cell layer. Electronmicroscopy confirmed the existence of single, unmyelinated and vesicle-containing nerve fibres located between the urothelial cells. Capsaicin treatment resulted in disappearance of both CGRP- and SP-IR fibres. In contrast, the number of NOS-IR fibres both in the musculature and suburothelium appeared to be unchanged. In conclusion, the sensitivity to capsaicin and the presence of free nerve endings primarily suggest a sensory role of fibres in the suburothelium/urothelium. Morphological data do not support the existence of NOS in the capsaicin-sensitive afferent nerve population in the rat lower urinary tract.

734.10

EFFECTS OF AMPA/KAINATE (LY215490) AND METABOTROPIC (MCPG) GLUTAMATERGIC RECEPTOR ANTAGONISTS, ON THE MICTURITION REFLEX IN RATS. M. Yoshiyama*, J.R. Roppolo, K.B. Thor & W.C. de Groat. Univ. of Pittsburgh, Sch. Med., Dept. Pharmacol., Pittsburgh, PA 15261 and Lilly Res. Lab., Indianapolis, IN 46285.

The role of glutamatergic transmission in voiding function was evaluated in unanesthetized decerebrate rats, by determining the effects of glutamatergic receptor (GluR) antagonists on urinary bladder and external urethral sphincter (EUS) EMG activity during cystometrograms. In spinal cord intact (SI) rats, LY215490 (LY), a competitive AMPA/kainate receptor antagonist, in small doses (1-3 mg/kg i.v.) decreased bladder contraction amplitude (BCA) and the EUS EMG by 15-35%; whereas in a large dose (10 mg/kg i.v.), it abolished bladder and EUS EMG activity. The intrathecal (i.t.) injection of LY (0.1-10 μg) decreased BCA by 30-100% and increased bladder capacity by 25-90%. Combined administration of LY (0.1 μg i.t.) and an NMDA receptor antagonist, MK-801 (MK, 1 μg i.t.) which individually had little effect on BCA, completely suppressed BCA. In chronic (3-4 weeks) spinal cord transected (ST) rats, LY (10 mg/kg i.v.) decreased BCA only 25% whereas it abolished EUS EMG activity. In ST rats, i.t. injection of LY (10 μg) decreased BCA by 50%. MK (1 mg/kg i.v.) administered prior to LY (10 mg/kg i.v.) abolished EUS EMG activity but had no significant depressant effect on BCA or on the depressant effect of LY on BCA. The intrathecal injection of (±)-α-methyl-4-carboxyphenylglycine (MCPG, 3-100 μg), a competitive metabotropic receptor ($mGluR_1$ and $mGluR_5$) antagonist, did not alter bladder activity but increased EUS EMG activity by 85% in SI rats. These data suggest that AMPA/kainate GluRs have a major role in the excitatory pathways controlling BCA and bladder capacity in SI rats. However, in ST rats, AMPA/kainate GluRs which are essential for EUS reflexes, appear to play only a minor role in bladder reflexes. Metabotropic receptors ($mGluR_1$ and $mGluR_5$) are also involved in control of EUS but not bladder activity.

734.11

MUSCARINIC FACILITATION OF TRANSMITTER RELEASE OPERATES VIA PRESYNAPTIC L-TYPE Ca^{2+} CHANNELS IN THE RAT URINARY BLADDER. G.T. Somogyi*, M. Tanowitz, G. Zernova and W.C. de Groat, Department of Pharmacology, University of Pittsburgh, Pittsburgh PA, 15261.

The effect of N and L type Ca^{2+} channel blockers were studied on muscarinic receptor mediated facilitation of ^{14}C-acetylcholine (ACh) and ^{3}H norepinephrine (NE) release in the rat urinary bladder strips. After prelabelling the strips with ^{14}C-choline and ^{3}H-NE, two periods of electrical stimulation (S1 and S2) were applied (20 Hz, and 0.25 ms) to increase the release of ACh and NE from nerve terminals. Control strips yielded an S_2/S_1 ratio of 0.88±0.06 for NE and 0.5±0.11 for ACh. Drugs were added 20 min before S_2. The Ca^{2+} channel activator, BAY-K 8644 (2μM) increased the release of NE and ACh (S_2/S_1 ACh: 6.44±2.41 and NE: 1.36±0.21). The N-type blocker omega conotoxin (CTX) (20 nM) reduced the S_2/S_1 values to 0.26 for NE but did not change that of ACh. The L-type channel blocker, nifedipine (1 μM) did not change the non-facilitated NE or ACh release. Transmitter release was facilitated by the cholinesterase inhibitor, eserine (5μM), which produced an S_2/S_1 value of 9.62±0.3 for ACh and 4.06±1.28 for NE. This facilitation was blocked by atropine (1 uM), but was not altered by CTX (20 nM) (S_2/S_1: ACh 9.02 and NE 3.41). In contrast, 1 μM nifedipine significantly reduced the eserine facilitated release of ACh and NE (S_2/S_1=2.05±0.53 and 1.48±0.27, respectively). These results suggest that N-type channels modulate the non-facilitated release of NE, whereas, the facilitated release of both ACh and NE is modulated by L-type channels. Previously we have shown that M1 facilitation utilizes a PKC dependent pathway. It is possible that PKC phosphorylates Ca^{2+} channels which are an essential link in muscarinic facilitation. Thus, inhibition either of M1 muscarinic receptors, PKC activity or L-type Ca^{2+} channels will prevent the facilitation of NE or ACh release. Supported by the NIH grant DK-45741.

734.12

CHEMICAL IRRITATION OR MECHANICAL DISTENSION OF THE URETHRA ELICITS ANO-EXCITATORY AND VESICO-INHIBITORY REFLEXES IN THE URETHANE-ANESTHETIZED RAT. M.A. Muhlhauser* and K.B. Thor, Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285

Transvesical infusion of dilute acetic acid (aa) in the intact or chronic spinal rat evokes a reflexive increase (5 fold) in anal sphincter (AS) activity *via* nociceptive fibers. Urinary bladder (BL) inhibition is frequently observed as well. Additionally, capsaicin (CAP) applied to urethra (UR) has been shown to inhibit BL. Because transvesical infusion exposes both BL and UR to aa, studies were conducted to determine whether it is specifically the irritation of BL, or UR, or both, that is triggering this ano-excitatory and vesico-inhibitory reflex. Rat BL were infused with saline or aa, BL pressures and AS EMG activity were recorded. Rats with BL isolated from urethra showed a modest (50%) increase in AS activity from aa infusion into BL alone but a 10 fold increase from infusion into the urethra. Infusion of aa into, or mechnical distension of, the isolated UR inhibited BL activity. CAP pretreatment (75mg/kg 4 days prior) significantly reduced AS activity elicited from urethral stimulation. Urethral pressure-AS activity response curves were generated by incremental pressure increases with either saline or aa. Increasing pressure with saline produced a linear increase in AS with BL inhibition occuring at 60 cmH2O. Acetic acid produced a steeper increase in AS with BL inhibition at 30 cmH2O. Finally, histology of BL tissue exposed to aa showed damage to epithelium but not underlying muscle. These studies suggest that rat urethral nociceptive afferent fibers are highly sensitive to chemical irritation or mechanical distension and mediate spinal ano-excitatory and vesico-inhibitory reflexes. These findings explain observed urologic phenomena in the rat and have clinical relevance to conditions associated with urinary retention accompanying prostatitis or perineal irritation.

734.13

DESENSITIZATION OF BLADDER SENSORY FIBERS WITH INTRAVESICAL CAPSAICIN IN HUMANS WITH DETRUSOR HYPERACTIVITY. F. Cruz *, M. Guimarães, C. Silva, M.E. Rio, M. Reis and A. Coimbra. Institute of Histology and Embryology, Dept. of Urology and Dept. of Neurology, Faculty of Medicine of Porto, 4200-Porto, Portugal.

Experimental studies have shown that detrusor hyperactivity very often follows the emergency of a strong spinal micturition reflex triggered by unmyelinated C-fibers. In the treatment of the urinary symptoms associated with this condition desensitization of C afferent fibers may constitute an alternative to anticholinergic drugs. With this purpose, capsaicin, the pungent extract of red peppers which is known to desensitize cutaneous C-afferents, is currently under investigation. We treated 10 patients with bladder hyperactivity associated with neurological disorders (8) pelvic radiotherapy (1) or idiopathic hypersensitive bladder (1). All patients gave informed written consent. A 1mM capsaicin solution in 30% alcohol was instilled through an urethral catheter and left in contact with the mucosa for 30 minutes. Patients mentioned a burning sensation immediately after capsaicin instillation that began waning slowly 15 minutes later. After 3 months day and night urinary frequency decreased about 50% in average and urge incontinence had disappeared. The average volume at which first desire to void occurred increased from 103±39ml to 218±113ml ($p<0.02$) and maximal cystometric capacity increased from 167±83ml to 350±211ml ($p<0.05$). Seven patients have been followed for 6 months and the clinical condition has deteriorated slightly in one of them. Average volumes of first desire to void and maximal cystometric capacity were 204±53ml ($p<0.001$) and 348±178ml ($p<0.05$), respectively. Three patients that completed one year of follow-up showed a pronounced deterioration of the clinical condition and the cystometric data returned to pretreatment levels.These findings suggest an important role for bladder afferent desensitization in the treatment of detrusor hyperactivity.

734.14

OPPOSITE EFFECTS OF DULOXETINE, A SEROTONIN (5HT) AND NOREPINEPHRINE (NE) RE-UPTAKE INHIBITOR, ON NOCICEPTIVE REFLEXES TO THE BLADDER AND URETHRAL SPHINCTER. K.B. Thor* and M.A. Katofiasc, Eli Lilly and Co., Indianapolis, IN 46285

5HT and NE systems are intimately associated with CNS control of LUT function. The present study has examined the effects of duloxetine (DUL) on bladder capacity and urethral sphincter EMG activity in chloralose-anesthetized cats. Cystometrograms (CMGs) were performed under conditions of saline infusion into the bladder or saline followed by dilute acetic acid (0.5%) infusion. Under saline conditions, bladder capacity was large (5ml) and sphincter EMG activity was low. During acetic acid infusion, bladder capacity was reduced (hyper-reflexia) and sphincter activity was increased. DUL had weak, non-significant effects on bladder capacity and sphincter activity during saline infusion. However, during acetic acid infusion, DUL (0.1 - 3 mg/kg) produced dose-dependent inhibition of bladder activity (*i.e.* increase in capacity) and facilitation of sphincter activity. Various experiments confirmed that the effects were mediated centrally through 5HT and/or NE receptors. These results indicate that monoaminergic systems can either inhibit or facilitate nociceptive-driven input depending on the efferent system studied. Since inhibition of the bladder and facilitation of the sphincter would be a coordinated, appropriate behavioral response under "fight or flight" conditions, it is tempting to speculate that the monoaminergic system "directs" nociceptive inputs to, or away, from specific efferent pathways to produce physiological changes useful in the context of "fight or flight" situations.

UROGENITAL REGULATION: SEXUAL FUNCTION

735.1

OXYTOCINERGIC INNERVATION OF THE SACRAL PARASYMPATHETIC NUCLEUS IN THE MALE RAT. Y. Tang, F. Giuliano*, O. Rampin, J.P. Rousseau and A. Calas. Dept of Urology and Lab. Chir. Exp., Fac. Méd. Paris-Sud, F-94270 Bicêtre: CNRS URA 1488, Univ. Paris 6, F-75005 Paris; CNRS URA 1449, Univ. Paris 6, F-75005 Paris.

The sacral parasympathetic nucleus (SPN) contains preganglionic autonomic neurons destined to the pelvic organs, and is the source of proerectile neurons. Oxytocin (OT) administered into the cerebral ventricles induces penile erection and yawning in the male rat. Oxytocinergic cell bodies are present in the paraventricular nucleus of the hypothalamus. We searched for the presence of oxytocinergic fibers in the SPN using retrograde labeling combined with immunocytochemistry. In adult anesthetized male rats, a solution of wheat-germ agglutinin-horseradish peroxidase (WGA-HRP, 25 % in distilled water) was applied on the central cut end of the left pelvic nerve. Rats were sacrificed two days later and serial sections of the lumbosacral spinal cord were treated with tetramethylbenzidine paratungstate and stabilized with diamidinobenzidine-cobalt complex to reveal retrograde-labeled SPN neurons. Immunocytochemistry was performed on the same sections using an anti-oxytocin antibody stained by the immunoperoxidase method. Retrograde labeled neurons were localized in the intermediolateral column at the L6 and S1 level of the spinal cord. They formed a dense and homogeneous population. At the same levels, OT-like immunoreactivity was associated with varicose fibers which were mainly found in the SPN, in the marginal zone and in the dorsal commissure. Rare OT-positive fibers were seen in the ventral horn. In the SPN, OT-immunoreactive fibers appeared in close apposition with retrograde-labeled neurons. At the ultrastructural level, OT-positive fibers containing DAB precipitates contributed varicose fibers which surrounded retrograde-labeled neurons and could make on them synaptic contacts. We conclude that oxytocinergic projections exist on SPN neurons. These results provide a morphological support for supraspinal control of oxytocin-mediated pelvic functions.

735.2

THE AUTONOMIC AND SENSORY INNERVATION OF THE RAT PROSTATE K.E. McKenna*, C.R. Georges, X.-B. Guan, and K.T. McVary. Depts. of Physiology and Urology, Northwestern University Medical School, Chicago, IL 60611

We have previously demonstrated that the autonomic innervation exerts a trophic influence on the prostate. Specifically, unilateral sympathectomy induced an ipsilateral atrophy, while unilateral parasympathectomy induced a contralateral hyperplasia.

The goal of the present studied was to identify the source of sensory and autonomic innervation of the prostate. Adult male rats were anesthetized and Fluorogold (2 µl, 4%) was injected unilaterally into the ventral prostate under aseptic conditions. After a 7-10 day survival period, rats were reanesthetized and perfused with fixative. The sympathetic chain, inferior mesenteric and major pelvic ganglia and dorsal root ganglia (DRG) were removed. Labeled neurons were counted in each ganglion.

The vast majority of labeled postganglionic neurons were located in the major pelvic ganglion, a mixed sympathetic-parasympathetic ganglion. Some labeled postganglionic neurons were observed in the sympathetic chain. Very few labeled neurons were seen in the inferior mesenteric ganglion. Labeled neurons were observed bilaterally in approximately equal numbers.

The afferent cell bodies from the prostate were localized predominantly in the dorsal root ganglia of L6, followed by L5 and S1. Approximately one third of the labeled DRG neurons were located in the T13-L2 segments. Labeling of sensory neurons was observed bilaterally.

735.3

ESTROGEN RECEPTOR-IMMUNOREACTIVE NEURONS ARE PRESENT IN THE FEMALE RAT LUMBOSACRAL SPINAL CORD. S.J. Williams* and R.E. Papka. Department of Anatomical Sciences, University of Oklahoma HSC, Oklahoma City, OK 73190.

Estrogens are gonadal steroids which stimulate secondary sex characteristics as well as growth, maturation and behavioral patterns in the CNS. Previous studies have examined potential sites of action of estrogen in the CNS by elucidating sites of neurons containing estrogen receptors, e.g., the hypothalamus, preoptic area and the amygdala. However, little attention has been directed to revealing estrogen receptor-containing neurons in the spinal cord, whether such neurons are involved in innervation of female reproductive organs, or if such neurons project information about reproductive organs to higher centers. A few studies have mentioned, somewhat in passing, the presence of estradiol-concentrating neurons in the spinal cord. Thus, we have undertaken a study of the location and distribution of estrogen receptor-immunoreactive (ER-IR) neurons in the female rat spinal cord by using immunohistochemistry.

Intact and ovariectomized rats were anesthetized, perfusion fixed and the spinal cords removed. The lumbosacral region was sectioned and immunostained with a rat monoclonal antibody (H222, Abbott Laboratories). ER-IR was observed mainly in the nuclei of neurons. ER-IR neurons were located predominately in the dorsal one-half of the spinal cord; specific sites containing ER-IR neurons include the dorsal horn, the sacral parasympathetic nucleus, lamina V, lamina X around the central canal and also extending into the lateral funiculus. The presence of ER-IR neurons in these regions suggest that neurons can be influenced by circulating estrogen. The ER-IR neurons could be involved in transmitting information either peripherally or to higher centers in the CNS. (Supported by NIH Grant NS22252 and Presbyterian Health Foundation.)

735.4

EXPRESSION OF ESTROGEN RECEPTOR (ER) IN NEURONS OF RAT SPINAL CORD, DORSAL ROOT GANGLIA (DRG) AND PELVIC AUTONOMIC GANGLIA (PG). B. Srinivasan, R.E. Papka, and K.E. Miller. Dept. Anatomical Sciences, University of Oklahoma HSC, Oklahoma City, OK 73190.

This study was directed to identify spinal cord neurons, sensory neurons and autonomic ganglionic neurons that could be responsive to estrogen. Ultimately, we want to use these data in assembling information regarding central and peripheral neural circuits related to uterine innervation. In situ hybridization histochemistry and immunohistochemistry were used to examine the presence and distribution of neurons producing estrogen receptor (ER) mRNA and ER protein respectively, in the lumbosacral cord levels L6-S1, L6-S1 DRG and PG of ovariectomized (ovex) and intact female rats. Unfixed spinal cords and ganglia were harvested from adult Sprague-Dawley rats, sectioned on a cryostat and sections processed for in situ hybridization histochemistry using oligonucleotides [48 bases] labelled with digoxigenin d-UTP. Immunohistochemistry was performed on sections of aldehyde-fixed spinal cords and ganglia using antisera from several different sources; sites of ER-immunoreactivity(IR) were visualized with an ABC reaction. Neurons with ER-IR nuclei were identified in the dorsal one-half of the spinal cord including preganglionic parasympathetic nuclei, lamina X and dorsal horn; labeled neurons were also present in the DRG and PG. ER mRNA expression was evident in many neurons in the spinal cord including those areas containing ER-IR neurons. Numerous neurons in the PG and DRG expressed ER mRNA; those in the DRG were mainly small and medium-size. Labeling was more evident in the ovex rats. These data suggest there are abundant neurons in the spinal cord and peripheral ganglia which could be responsive to circulating estrogen. (Supported in part by NIH grant NS-22526).

735.5

IDENTIFICATION OF 5-HT RECEPTOR SUBTYPES INVOLVED IN SEXUAL REFLEXES IN THE SPINAL CORD OF MALE RAT. X.-B. Guan*, K.T. McVary and K.E. McKenna. Depts of Physiology and Urology, Northwestern University Medical School, Chicago, IL 60611

We have previously demonstrated that spinal sexual reflexes are under the control of a descending inhibitory 5-HT pathway from the nucleus paragigantocellularis (nPGi). However, the specific receptor mechanism involved in this process has not been identified. The purpose of this study was to identify the 5-HT receptor subtype(s) involved in the descending control of sexual reflexes. Primers for 5-HT receptor subtypes 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1F} were designed and synthesized. A reverse transcription followed by polymerase chain reaction (RT-PCR) for these three 5-HT receptor subtypes were conducted to observe the expression of these receptors at the message level in the lumbosacral segments of the spinal cord. Male Sprague-Dawley rats were used in this study. The spinal cord was dissected into L_{1-2}, L_{3-4}, and L_{5-S1} segments and then further dissected into dorsal and ventral portions. Total RNA was used for reverse transcription. The PCR products were resolved on a 2% MetaPhor agarose gel stained with ethidium bromide. The expected size of products for all three receptor subtypes were obtained (342 bp for 1A, 244 bp for 1B and 494 bp for 1F, respectively). Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was co-amplified as an internal standard. The density ratio of the 5-HT receptor and G3PDH was used to quantitate the mRNA amount. While the 1B receptor has been clearly identified in the lumbosacral segments of the spinal cord, the distribution of the other receptor subtypes and the control mechanism are still under the investigation.

735.6

BIOCHEMICAL EVALUATION OF NITRERGIC INNERVATION OF THE RAT PENIS. J. Bernabé*, A. Meulemans, O. Rampin and F. Giuliano. Lab. Neurobio. Végétative, I.N.R.A., F-78352 Jouy-en-Josas, Lab. Biophysique, Hôpital Bichat, F-75018 Paris and Lab. Chir. Exp., Fac. Médecine Paris-Sud, F-94270 Bicêtre.

In vitro and in vivo experiments have demonstrated the role of nitric oxide (NO) in the relaxation of smooth muscle fibers of penile erectile tissue. Nitric oxide synthase (NOS) catalyzes the formation of NO and L-citrulline from L-arginine. NO reacts with oxygen to produce nitrite (NO_2-) and nitrate (NO_3-). Nitrergic axons to the penis are derived from the major pelvic ganglion and conveyed by the cavernous nerve. Using biochemical tools, we quantified the nitrergic mediation of neural origin in the corpora cavernosa. Cavernous nerves were cut bilaterally in 35 adult male rats (CNx) and 35 rats were sham operated (Co) and allowed a 7-day recovery period. Total amounts of NOS (µg) and NOS specific activity (pM/hour/mg NOS) were measured by quantification of [3-H] citrulline (Bredt et al., 1990) in homogenates from the corpora cavernosa. NOS specific activity in the cerebellum was used as the reference. NO_2- + NO_3- assays were performed using a sensitive method of capillary electrophoresis with direct U.V. detection (Meulemans and Delsenne, 1994). Total amounts of NOS were comparable in Co and CNx rats (33.6 ± 2.1 and 28.6 ± 4.1, respectively). In Co rats, NOS specific activity was 0.025 ± 0.004 in the corpora, and 0.071 ± 0.019 in the cerebellum. In CNx rats, NOS specific activity was decreased by 20 % in the corpora (0.020 ± 0.004), but not in the cerebellum (0.064 ± 0.012). NO_2- + NO_3- levels were 1.23 ± 0.12 in the corpora of Co rats and 0.90 ± 0.06 in the corpora of CNx rats (a decrease of 27 % relative to Co rats). These results indicate that neuronal NOS represents a small part of total NOS in the corpora. Nevertheless, a decrease in NOS specific activity in CNx rats suggests that neuronal NOS plays a significant physiological role. This hypothesis is reinforced by the fact that the total amount of NO_2- + NO_3- decreased in the corpora cavernosa in CNx rats. Moreover, from comparison of total amounts of NOS and NOS specific activity in control and denervated corpora, we infer that neuronal and non-neuronal NOS display different specific activities.

735.7

CYTOCHROME OXIDASE STAINING IN THE RAT MAJOR PELVIC GANGLION (MPG). W. G. Dail*, R. Galindo, F. Harji, L. Leyba and V. Barba. Dept. of Anatomy, Univ. New Mexico, School of Medicine, Albuquerque, NM 87131

Pelvic autonomic neurons display a variety of neurochemical and functional differences, which in part reflect the diverse reproductive and eliminative organs they regulate. This study localizes cytochrome oxidase (CO) activity to determine if the heterogeneity in pelvic neurons extends to a marker of metabolic activity. A distinct topography is apparent in that neurons which stain lightly for CO are localized in the ventral portion of the MPG near the entry of the hypogastric nerve (HN), while darker neurons predominate in the dorsal pole. The most intensely reactive neurons are located in a longitudinal band in the central region of the MPG. Terminals (verified by their disappearance after cutting the HN) around lightly stained neurons vary in size and are foci of intense CO activity, whereas terminals surrounding neurons in the dorsal pole are less apparent. Topographic relationships and immunoreactivity for tyrosine hydroxylase suggest that large cells with relatively low CO activity are adrenergic neurons while penile neurons (located by retrograde tracer) are moderately active. These findings suggest that the varied basal metabolic activity of neurons in the MPG mirrors the level of synaptic activity needed to regulate respective pelvic organs. Supported by NIH RO11983-11.

735.8

HORMONAL CONTROL OF CHOLINERGIC, NORADRENERGIC AND PEPTIDERGIC TRANSMITTERS IN AUTONOMIC GANGLIA. R. W. Hamill*, V. May, and K. M. Braas. Departments of Neurology, and Anatomy & Neurobiology, Univ. of Vermont College of Medicine, Burlington, VT 05405.

Hormonal influences regulate biochemical and morphological features of neurons. Previous studies indicate that androgens influence the neurochemistry of the hypogastric ganglia (HG). The current studies using adult Sprague-Dawley rats, examine the effects of castration on noradrenergic, cholinergic, and peptidergic systems in the major pelvic ganglia (MPG), sexually dimorphic ganglia innervating the urinary bladder and reproductive organs in the pelvis. Adult castration alters the catalytic activities of Tyrosine Hydroxylase (TH), the rate limiting enzyme in noradrenaline biosynthesis and a marker of noradrenergic adaptability, and Choline Acetyltransferase (ChAT), a marker of cholinergic components of autonomic ganglia. Four weeks following castration TH and ChAT activities are 25% and 50% of control, respectively. At 8 and 12 weeks post-castration, ChAT levels remain at 50%, whereas TH activity gradually declines reaching a nadir of 15% of control by 12 weeks. Castration also alters the levels of vasoactive intestinal peptide (VIP), which is colocalized with ChAT in MPG. VIP levels are reduced approximately 25% at 4 weeks following surgery. In all paradigms, testosterone replacement therapy fully restores TH, ChAT, and VIP levels. These studies indicate that the biosynthesis of noradrenergic, cholinergic, and peptide neurotransmitters in the adult MPG are regulated by testosterone. Taken together with previous studies of the HG, the results indicate that parasympathetic and sympathetic ganglia involved in reproductive function respond to gonadal steroids during adulthood. (Supported by NS22103-RWH; HD27268 and NS01636-VM; AHA94015540-KMB).

735.9

CLOMIPHENE CITRATE (CC) IMPROVES SPERM MOTILITY IN SPINAL CORD INJURED (SCI) MEN. C.M. Lynne* and N.L. Brackett. Dept. Urology, The Miami Project to Cure Paralysis, and Dept. of Neurological Surgery, U. Miami Sch. Med., Miami, FL 33136

The majority of men with SCI are in the parenting age group. Most of these men suffer from low numbers of motile sperm. This pilot study assessed the efficacy of CC for improving sperm motility in SCI men. Four volunteer SCI men had monthly semen analyses for 6-12 months prior to CC ("-CC"), and then again for 6-12 months during treatment with CC, ("+CC") given as 25 mg orally every other day. The following semen parameters were measured in the "-CC" and in the "+CC" conditions: (a) total number of sperm per ejaculate, (b) number of motile sperm per ejaculate, (c) percent motile sperm per ejaculate, (d) total number of sperm with rapid linear motion per ejaculate, (e) the percent of motile sperm per ejaculate with rapid linear movement, and (f) the percent motile sperm in the antegrade fraction only. ANOVA was used to evaluate the statistical significance of any changes in the "-CC" and "+CC" conditions. All measures of sperm motility improved significantly with CC treatment. There was no difference in the total number of sperm per ejaculate with CC treatment. Based on this small group of subjects, it appears that CC may improve sperm motility in SCI men. To our knowledge, no treatment regimen has heretofore been shown to have any efficacy in these subjects. A larger study is needed evaluate the effect in more SCI men and to evaluate the effect on fecundity.

735.10

SEMINAL PLASMA OF SPINAL CORD INJURED (SCI) MEN INHIBITS SPERM MOTILITY OF NORMAL MEN N.L. Brackett*, R.C. Davi, O.F. Padron, and C. M. Lynne, The Miami Project to Cure Paralysis, Dept. of Neurological Surgery, Dept. of Urology, Univ. of Miami Sch. Med., Miami, FL 33136.

SCI men have sperm of normal concentration but poor motility (Brackett et al., J Urol, 151:114-119, 1994). No single factor has been identified as a cause for this condition. **Objective:** The present study investigated if the seminal plasma of SCI men contributes to their poor sperm motility. **Method:** Aliquots of washed sperm from normal men (n=13) were mixed either with seminal plasma from SCI men or with seminal plasma from other normal men. Similarly, aliquots of washed sperm from SCI men (n=13) were mixed either with seminal plasma from normal men or with seminal plasma from other SCI men. **Results:** Percent of motile sperm in normal men decreased significantly, from 72% to 45% ($p<0.001$), when mixed with seminal plasma from SCI men. Percent of motile sperm in SCI men was significantly higher when mixed with seminal plasma of normal men (42%), than with seminal plasma from SCI men (29%) ($p<0.05$). **Conclusions:** Seminal plasma of SCI men appears to contribute to their poor sperm motility. There may be toxic factors present and/or nutritive factors absent which, when mixed with sperm during ejaculation, act to impair sperm motility.

735.11

STIMULATION OF THE DORSAL NERVE OF THE PENIS INCREASES C-FOS EXPRESSION IN SPINAL NEURONS IN THE RAT. O. Rampin*, S. Gougis, F. Giuliano and J.P. Rousseau. Lab. Neurobio. Fonctions Végétatives, I.N.R.A., F-78352 Jouy-en-Josas.

Physiological evidence of a reflex loop mediated at the spinal level and controlling reflexive penile erection has been reported in the rat (Rampin et al., Neurosci. Lett. 1994, 180:138). The dorsal nerve of the penis (DNP) represents the afferent limb of this reflex. In the present study, c-Fos immunohistochemistry was used to identify neurons in the spinal cord that receive afferent input from the DNP. Electrical stimulation of the left DNP (6V, 5 Hz, 0.1 ms) was applied for 10 minutes in anesthetized male adult rats. Segmental distribution of c-Fos positive neurons was studied over the T13 to S1 levels. Average numbers of positive neurons per spinal segment and differences in distribution of positive neurons in specific areas of the spinal cord were calculated. In control rats, c-Fos positive neurons were present mainly at the L5-S1 levels. They were located in the dorsal gray commissure (DGC, 35.7±2.1 %), and bilaterally in the intermediolateral cell column (sacral parasympathetic nucleus, SPN, 6.3±1.1 %) and in the dorsal and ventral horns (DH, 56.8±2.7 %; VH, 0.8±0.3 %). Following acute T8 spinalization, DNP stimulation elicited an increase of c-Fos positive neurons over the L5-S1 levels, compared to control rats. Percent distribution was increased in SPN (12.2±0.8 %, $p < 0.001$) and VH (2.2±0.7 %), decreased in DH (45.2±2.7 %, $p < 0.05$) and unchanged in DGC (39.9±2.2 %). These findings provide a morphological support for a reflex loop mainly organized at the L5-S1 levels of the spinal cord, and responsible for penile erection. Localization of c-Fos positive neurons outside of the SPN suggests the hypothesis of the presence of interneurons between afferent terminations and proerectile preganglionic parasympathetic neurons, and/or possible sites for supraspinal projections modulating reflexive erections. Furthermore the increase in c-fos expression observed after acute T8 spinalization supports the presence of a supraspinal inhibitory control exerted on the lumbosacral circuitry responsible for reflexive erection (Sachs and Garinello, J. Comp. Physiol. Psychol. 1980, 94:530).

RESPIRATORY REGULATION: AMINO ACID TRANSMITTERS

736.1

THE ROLE OF BRAINSTEM RETROTRAPEZOID NUCLEUS (RTN) METABOTROPIC RECEPTORS IN THE PROLONGED STIMULATION OF RESPIRATION PRODUCED BY RTN INJECTION OF GLUTAMATE. Aihua Li, and Eugene E. Nattie*. Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756-0001

Stimulation of metabotropic glutamate receptors (mGluRs) in the RTN of chloralose-urethane anesthetized rats by the mGluR agonist (1S,3R)-aminocyclopentanedicarboxylic acid (1S,3R-ACPD) increases integrated phrenic amplitude (PNA) for > 60 min. This long-lasting PNA stimulation can also be mimicked by the long duration (60 sec) injection of glutamate. Here we show the mGluR antagonist (±)-α-methyl-4-carboxyphenylglycine (±-MCPG) can block both of these long lasting effects. Using multi-barreled micropipettes, we first identified RTN sites associated with respiration by observation of glutamate induced short-lived PNA stimulation. In group 1, five rats received only mGluR antagonist (±)MCPG (10nl, 10mM); in group 2, six rats received mGluR antagonist (±)-MCPG (10nl, 10mM) followed by the agonist 1S,3R-ACPD (10 nl, 1mM) injection every 30min until the PNA response to the agonist recovered. In group 3, five rats received 6 injections of inactive mGluR antagonist (-)-MCPG (10nl, 10mM) followed by agonist 1S,3R-ACPD (10nl, 1mM) after 30min. In group 4, five rats received (±)-MCPG followed by long duration (60 sec) injection of glutamate (10nl, 100mM) every 30 min until the PNA response to glutamate was evident. The active antagonist (±)-MCPG had no significant effect on PNA but it blocked a) subsequent 1S,3R-ACPD induced PNA stimulation for 99±11min and b) the PNA response to 60 sec glutamate injection for 66±6 min. The inactive form (-)-MCPG had no effect on ACPD-induced PNA stimulation. We conclude: 1) RTN mGluRs are involved in respiratory control, 2) they are not active in eupnea and 3) their stimulation may require prolonged glutamate release. (Supported by HL 28066)

736.2

GLUTAMATE SYNTHESIS BY EXCITATORY RESPIRATORY NEURONS IN THE VENTROLATERAL MEDULLA. P.M. Pilowsky*, Q.-J. Sun, I.J. Llewellyn-Smith, L.F. Arnolda, J.P. Chalmers and J.B. Minson. Dept of Medicine and Centre For Neuroscience, Flinders Medical Centre, Bedford Park 5042, South AUSTRALIA.

Excitatory respiratory pre-motoneurons are located in the ventral respiratory group (VRG) of the brainstem, while inhibitory pre-motoneurons are found in the Bötzinger region. Together they regulate the activity of phrenic motoneurons. In this study we aimed to determine if these brainstem respiratory neurons are immunoreactive for the glutamate synthesizing enzyme, phosphate activated glutaminase (PAG). Respiratory neurons in the ventrolateral medulla of pentobarbitone-anaesthetised Sprague-Dawley rats were labelled by intracellular injection of Neurobiotin (Vector, CA). After histochemical processing and immnuohistochemistry, sections of brainstem were examined by fluorescence and light microscopy. Four types of respiratory neurons were identified. 1) Six inspiratory neurons in the VRG: five PAG immunoreactive and one PAG negative. 2) Six expiratory neurons in the VRG: five neurons were PAG immunoreactive and one PAG negative. 3) Five neurons in the Bötzinger area: four PAG negative and one PAG immunoreactive. 4) Seven ambigual motoneurons that were all PAG immunoreactive. Our results support the use of PAG as a marker for glutamatergic respiratory neurons since most of the excitatory VRG neurons contained moderate to strong levels of immunoreactivity while most Bötzinger neurons, which are believed to be inhibitory, contained none. These findings also suggest that glutamate is synthesized and released as a neurotransmitter by excitatory respiratory neurons.

736.3

COMPARISON OF GAD- AND GABA- IMMUNOREACTIVE NERVE TERMINALS IN THE RAT PHRENIC MOTOR NUCLEUS Susan M. Murphy*, Paul M. Pilowsky and Ida J. Llewellyn-Smith, Dept. of Medicine, Flinders Medical Centre, Bedford Park, South Australia, 5042.

γ-aminobutyric acid (GABA) mediates synaptic inhibition of phrenic motoneurons during expiration. About 30% of nerve terminals that make synapses or direct contacts with phrenic motoneurons are immunoreactive for GAD, the GABA synthetic enzyme, whilst only about 18% of inputs identified by post-embedding immunogold are GABA-positive (SN 1994, Poster 231.8). In this study, we examined the distribution of these two markers in adjacent pairs of ultrathin sections containing retrogradely-labelled phrenic motoneurons. Both sections were labelled for GAD-immunoreactivity by pre-embedding immunocytochemistry, and one section of each pair was subsequently processed for post-embedding immunogold labelling for GABA. We examined terminals that synapsed on or directly contacted phrenic motoneurons, as well as other non-synaptic terminals. Many terminals containing high densities of gold particles also proved to be GAD-positive. However, not all GAD-immunoreactive nerve terminals were strongly labelled for GABA. Comparisons of the density of gold labelling over GAD-positive and GAD-negative terminals suggest that there is overlap in the frequency distributions of the two populations. Assuming GAD is itself a reliable marker for GABAergic terminals, we must conclude from these and our previous results that in this part of the spinal cord, post-embedding immunocytochemistry for GABA alone does not provide a sufficient basis to discriminate between GABAergic and non GABAergic terminals that provide synaptic input to phrenic and other motoneurons.

736.4

PROXIMAL SYNAPSES IN THE LUNG C-FIBER AFFERENT PATHWAY: POTENTIAL TRANSMITTER ROLE OF EXCITATORY AMINO ACIDS (EAAs). C.G. Wilson*, Z. Zhang, A.C. Bonham. Univ. California, Davis, Davis, CA 95616.

We previously identified synapses in the lung C-fiber afferent pathway in the mediocaudal commissural nucleus tractus solitarius (cNTS) which were essential for C-fiber evoked apnea and rapid shallow breathing (RSB). Here we examined the role of EAAs as potential neurotransmitters. Phrenic nerve activity, blood pressure and heart rate were continuously recorded in urethane-anesthetized, paralyzed, and ventilated rats. C-fibers were stimulated by injecting phenylbiguanide (PBG; 0.05-0.1ml, 50μg/ml) in the right atrium (RA) and left ventricle (LV). In some rats we cut both vagi below the diaphragm and denervated the baroreceptors and chemoreceptors. Medial cNTS was screened for vagally-evoked units with a 7-barrel electrode containing 2% pontamine dye in 0.5M NaAc for extracellular recording, EAA agonists and antagonists for iontophoresis, and normal saline for balancing currents. RA PBG (n=25) evoked immediate increases in unit activity: (1±2 to 16±18Hz) (p=.0004) for 17±20s, that coincided with apnea or RSB. In 9 cells RA PBG compared to LV PBG produced greater increases in activity (14±16 vs 8±6Hz; $p < .04$) for longer durations (8±6 vs 3±4s; p=.01)(n=9). The EAA agonists NMDA (-8 to -30nA; 100mM) and QUIS (-8 to -60nA; 100mM) evoked dose-dependent increases in unit activity. NMDA (24±7nA) and Quis (29±15nA) increased unit activity from 0.5±0.8 to 7±2Hz and from 0.4±0.4 to 11±5Hz, respectively. The NMDA receptor antagonist, AP5 (-90 nA, 100 mM) in doses that blocked NMDA- but not QUIS-evoked increases in unit activity, did not alter PBG-evoked increases in activity. Activation of both NMDA and nonNMDA receptors stimulates cNTS neurons in the C-fiber afferent pathway, but NMDA receptors are not involved in neurotransmission. Supported by NIH HL48584.

736.5

DESENSITIZATION OF GLUTAMATE RECEPTORS MODULATES RESPIRATORY DRIVE IN FETAL SHEEP. J.M. Bissonnette*, A.R. Hohimer and S.J. Knopp. Department of Obstetrics and Gynecology, Oregon Health Sciences University, Portland, OR 97201.

Blockade of non-N-methyl-D-aspartate (NMDA) receptors in the medulla of fetal sheep inhibits respiratory output (FASEB J. 9:A836, 1995). Both kainate and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) preferring non-NMDA glutamate receptors rapidly desensitize in the presence of ligand (eg. Neuron 11:1069, 1993). Studies were carried out in unanesthetized fetal sheep to examine the effect of preventing desensitization on respiratory drive measured from transient pressure changes in a tracheal catheter. Following a control period cyclothiazide which has been shown to block desensitization of AMPA preferring receptors (Ibid.) was instilled in the cerebrospinal fluid of the fourth ventricle (75 μg). Breath to breath analysis showed that the slope of inspiration (rate of pressure change) increased by 62 to 221 percent from baseline values which ranged from 7.8±0.2 (SEM) to 13.4±0.2 $mmHg \cdot sec^{-1}$. There was a variable effect on respiratory timing either no change or a shortening of both inspiratory and expiratory time. The effect lasted between 30 and 60 minutes after a single injection. The benzothiadiazides have carbonic anhydrase inhibitory properties but equivalent doses of acetazoleamide did not result in similar changes in respiratory output. These experiments suggest that AMPA preferring glutamate receptors at respiratory related neurons undergo desensitization and modulation of this characteristic results in an increase in respiratory output. (Supported by HL 38039)

736.6

CENTRAL ADMINISTRATION OF ASPARTIC ACID AFFECTS VENTILATION AND O_2 CONSUMPTION DIFFERENTLY IN MALE & FEMALE RATS. E. H. Schlenker*, S. R. Inamdar & D. Rivera-Hopkins. Univ. of S. D. School of Medicine, Vermillion, SD 57069

Aspartic acid (AA) injected subcutaneously induces a long lasting depression of ventilation (VE) in male but not in female rats. Systemic administration results in uptake of AA into the arcuate nucleus-median eminence area of the hypothalamus. In this study AA in doses of 100 & 200 nmol/0.5 μl relative to vehicle (CSF) injected into the arcuate nucleus of male rats resulted in a depression of $\dot{V}E$ and O_2 consumption ($\dot{V}O2$). Similar treatment of female rats caused in a depression of $\dot{V}O2$ without an effect on $\dot{V}E$. Intracerboventricular (ICV) injection of 3 μl of AA in doses of 0.5, 1 & 2 ng into the lateral ventricles of females affected $\dot{V}E$ & $\dot{V}O2$ similar to the arcuate injection, but resulted in an increase of $\dot{V}O2$ in male rats with no effect on $\dot{V}E$. In both sets of experiments the effects of AA were long lasting and reversible with time. We conclude that 1. AA injected into the arcuate nucleus has different effects on $\dot{V}E$ in male and female rats, although $\dot{V}O2$ is depressed in both groups; 2. depression of $\dot{V}E$ in males is not nonspecific (comparing arcuate and ICV results); & effects of AA on $\dot{V}E$ are not always coupled with similar effects on $\dot{V}O2$.
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736.7

DIFFERENTIAL EFFECTS OF $GABA_A$ RECEPTOR ANTAGONISTS SUGGEST THE EXISTENCE OF A POTENT GAIN-CONTROL MECHANISM IN BULBOSPINAL RESPIRATORY NEURONS. Z. Dogas, E. Stuth, F. Hopp, D. McCrimmon, and E. Zuperku*. Zablocki VA Med. Ctr., Med. Col. of WI, Milwaukee, WI 53295 and Northwestern U., Chicago, IL 60611.

The role of $GABA_A$ receptors in the control of inspiratory (I) and expiratory (E) bulbospinal neurons (BSNs) was studied *in vivo* using pressure microejection while recording single unit activity in the caudal ventral respiratory group of anesthetized, ventilated, paralyzed dogs. Bicuculline (BIC; 0.5 mM), picrotoxinin (PTXN; 5 mM), and artificial cerebral spinal fluid (aCSF) were ejected from multibarrel glass micropipettes and volumes were measured via meniscus changes (50X microscope). The competitive antagonist, BIC, (3-35 pmol) produced 100-200% increases in the spontaneous phasic discharge frequency (F_n) without increasing activity during the normally silent phase of these neurons. The noncompetitive antagonist, PTXN, (40-720 pmol) produced slight increases (≈10% of control) in phasic F_n, however, during the silent phase, there was a dose-dependent increase in F_n (EBSNs: 52.5±18.9%- IBSNs: 36.7±4.9% of peak phasic control F_n). Thus, BIC amplified the underlying phasic pattern, while PTXN antagonized the silent phase inhibition. Phrenic nerve activity and I and E durations were not altered by these microejections. These studies suggest the existence of a BIC-sensitive, PTXN-insensitive gain-control mechanism in both IBSNs and EBSNs which may be mediated via $GABA_A$ receptors with novel pharmacology, and that the inhibition which produces the normally silent phase in these neurons is primarily mediated by $GABA_A$ receptors. Supported by VA Med. Research Funds.

736.8

BLOCKADE OF $GABA_A$ BUT NOT GLYCINE RECEPTORS IN THE PRE-BÖTZINGER REGION PRODUCES AN AUGMENTED OR GASP-LIKE PATTERN IN THE PHRENIC NEUROGRAM IN CATS. I.C. Solomon*, J.E. Melton, N.H. Edelman, and J.A. Neubauer. Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ.

Progressive brain hypoxia results in a shift from respiratory depression to gasping. Previous work from our laboratory has identified a region in the pre-Bötzinger (pre-Böt) complex in which chemical activation of neurons evokes an augmented or gasp-like pattern in the phrenic neurogram. To test the role of disinhibition in the production of gasping, we examined the effects of blockade of $GABA_A$ and glycine receptors in this region on respiratory patterning. In chloralose-anesthetized, vagotomized, and paralyzed cats, we identified a region in the pre-Böt complex that produced a gasp-like pattern in the phrenic neurogram when chemically stimulated unilaterally with DL-homocysteic acid (DLH; 10 mM; 21 nl). After recovery, we recorded phrenic neurogram (PN) responses to unilateral microinjection of bicuculline methiodide (BIC; 5 mM; 42 nl) and/or strychnine (STR; 2 mM; 42 nl) in the same region. Microinjection of BIC into 8 of 9 sites produced either an augmented (n = 4) or gasp-like (n = 4) pattern in the PN. In many of these sites, a series of eupneic breaths was interspersed between each augmented or gasp-like burst. In contrast, microinjection of STR into 5 sites was ineffective in changing the pattern of PN activity. Further, microinjection of STR 20-30 minutes after microinjection of BIC (n = 5) produced no additional change in PN activity. These findings suggest that $GABA_A$-mediated, but not glycine-mediated, inhibition of the pre-Böt region may play a role in suppression of gasping during eupnea. Supported by HL16022, HL07467, HL44678.

736.9

MK-801 ALTERS THE RESPIRATORY RESPONSE TO HYPOXIA IN ADULT RATS. S.K. Coles*, M.F. El-Khatib, and T.E. Dick. Dept. of Medicine, Div. of Pulmonary and Critical Care Medicine, Case Western Reserve Univ., Cleveland, OH 44106

Blocking NMDA receptor channels with the non-competitive NMDA antagonist, MK-801, prolongs T_I without changing T_E. Reversible bilateral inhibition of cellular activity (10mM muscimol) in the dorso- or ventrolateral pons produces a similar breathing pattern and, further, selectively attenuates the depressant effect of acute, severe hypoxia on respiration (i.e., post-hypoxic frequency depression, PHFD). The purpose of this study was to determine if blocking NMDA receptor channels would selectively affect PHFD.

Adult, male Sprague-Dawley rats (360-525 g) were anesthetized with Equithesin, vagotomized, paralyzed, and ventilated with 100% O_2. Animals were exposed to 8% O_2 for 30 s (n=6) or for 60s (n=1) before and after intravenous injection of MK-801 (0.3mg/kg). Phrenic nerve activity was recorded to determine respiratory cycle. Blood pressure, air flow, pCO_2, and body temperature were monitored.

During hypoxia before MK-801, respiratory frequency increased initially then decreased, and it decreased further following hypoxia, i.e., PHFD. These changes in frequency were primarily due to changes in T_E; T_I remained relatively unchanged. Injection of MK-801 at this low dose elicited minimal changes in respiratory frequency (n=4). However, in 3 animals, respiratory frequency decreased 30%. MK-801 altered the respiratory response to hypoxia in all animals. In 6 animals, respiratory frequency decreased rather than increased; this was primarily due to an increase in T_I rather than in T_E which changed inconsistently. Following hypoxia, T_E did not increase, and PHFD was attenuated in all rats.

We conclude that NMDA receptors have a significant role in determining the frequency response to hypoxia. Thus, blocking NMDA receptors altered either directly or indirectly the role of the lateral pons in mediating PHFD.

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736.10

EFFECTS OF NBQX ON RESPIRATION IN ADULT RATS. Caroline A. Connelly*, Department of Surgery, University of California-Davis, Sacramento, CA 95817

N-methyl-D-aspartate (NMDA) receptors are significantly involved in respiratory pattern generation in adult rats (Connelly et al., *Brain Res.*, 596: 99-110, 1992), but not neonatal rat brainstem/spinal cords in *vitro*. Non-NMDA receptor activation is critical for maintaining respiratory rhythms *in vitro* (Funk et al., *J. Neurophysiol.* 70(4):1497-515, 1993). The present study examined the role of non-NMDA receptors in the generation of respiratory rhythm in spontaneously breathing adult rats.

6-Nitro-7-sulphamoylbenzo(f)quinoxaline-2,3-dione (NBQX) is an AMPA/kainate receptor antagonist that crosses the blood-brain barrier. NBQX (40 mg/kg, i.v., pH= 7.45-7.49 in 5.5% glucose) was administered to anesthetized Sprague-Dawley rats while diaphragm EMG activity and arterial pressure were monitored. Respiratory frequency significantly decreased ($p<0.01$, n=6); inspiration and expiration were both prolonged. Significant decreases in arterial pO2 and pH indicated that NBQX depressed ventilation. Arterial pCO2 increased in 5/6 rats. Changes in arterial pressure were insignificant. When the NMDA receptor-channel blocker, MK-801 (1mg/kg, i.v.), was injected 5-15 min after NBQX, respiratory frequency decreased further and apnea occurred within 45 s to 1 min. 5/6 rats had apneustic breaths (1.5 to 18 s duration) immediately prior to the onset of apnea. The rats were artificially ventilated until spontaneous breathing resumed after 7-85 min.

These data indicate that non-NMDA receptor activation is significantly involved but not necessary for respiratory rhythm generation in adult rats. These results support the hypothesis that combined activation of NMDA and non-NMDA receptors underlies excitatory transmission necessary for respiratory rhythm generation. (NBQX was generously provided by Novo Nordisk).

737.1

SEROTONIN IN THE DORSAL RESPIRATORY GROUP DECREASES HYPOGLOSSAL (XII) NEURAL ACTIVITY. M.A. Douse*, E.J. Puglisi and D.P. White. Dept. of Medicine, UCHSC, and Respiratory Care, VAMC, Denver, CO 80220.

Serotonin (5HT) is known to increase XII motoneuron activity when applied directly into the XII motor nucleus, but nothing is known concerning 5HT modulation of XII activity via other integration sites in the brainstem. We determined the effects of 5HT and methysergide (broad 5HT antagonist) pressure microinjection into the dorsal respiratory group (DRG; 25-100 nl; pH=7.2-7.4) on XII whole nerve activity in 6 decerebrate, vagotomized, paralyzed and artificially ventilated cats. The DRG was located by recording inspiratory activity at the appropriate co-ordinates and confirmed by antidromic activation from the C3 spinal cord. Surprisingly, pressure microinjection of 0.005 mM 5HT into the ipsilateral DRG resulted in a volume dependent bilateral decrease in XII whole nerve activity, measured as the inspiratory peak height of XII integrated neural activity (time constant=100 msec). XII neural activity ipsilateral to the injection site significantly decreased to 85.0 ± 5.4 % (25 nl); 74.2 ± 9.1 % (50 nl); and 50.4 ± 10.5 % (100 nl) of control values (n=5; all $p<0.05$). Contralateral XII activity decreased to 92.7 ± 2.8 % (25 nl; $p>0.05$); 91.2 ± 4.2 % (50 nl; $p>0.05$); and 74.0 ± 8.3 % (100 nl; $p<0.05$) of control (n=5). Pressure microinjection of 1.0 mM methysergide (100 nl) into the DRG had no effect on XII neural activity (97.6 ± 4.4 % of control; n=5) but did result in a full (99.5 ± 3.9 % of control; n=3) or incomplete (decreased to 53.0 ± 28.8 % of control; n=2) antagonism of subsequent pressure microinjection of 0.005 mM 5HT (100 nl). We conclude that exogenous 5HT in the DRG decreases XII neural activity. The data further suggest that the DRG may be an important site of 5HT modulation of respiratory drive to the XII motoneurons.

737.2

EFFECT OF SEROTONERGIC 1A AND 1B AGONISTS ON HYPOGLOSSAL MOTONEURONS. S. Okabe and L. Kubin*. Center for Sleep and Respiratory Neurobiology, Departments of Medicine and Animal Biology, University of Pennsylvania, Philadelphia, PA 19104.

Serotonergic effects on hypoglossal (XII) motoneurons have been implicated in sleep-related decrements in upper airway tone that may lead to airway obstructions. So far, serotonergic excitation, presumably mediated by type 2 receptors, was studied in some detail. However, the XII nucleus also contains type 1 receptors whose role in the control of XII motoneurons remains largely unknown. Our goal was to assess the effects of microinjections into the XII nucleus of 1A (8-OH-DPAT) and 1B (CGS12066) receptor agonists on XII nerve activity.

In 25 urethane anesthetized, paralyzed, vagotomized and artificially ventilated rats, we recorded XII nerve activity bilaterally. Microinjectionsof 8-OH-DPAT (0.1-10 mM) or CGS12066 (0.02-5.0 mM) were performed (10 nl, saline as vehicle) in histologically verified sites. At the highest concentrations used, both drugs suppressed XII nerve activity by about 20%. The suppression occurred within 2 min after the injection and lasted for at least 20 min. At a concentration of 0.1mM for 8-OH-DPAT and 0.02 mM for CGS12066, there was only a residual suppression of less than 5%.

Given that the affinity of 8-OH-DPAT to 1A receptors is at least 20-200 times higher than that of CGS12066 to either 1A or 1B receptors, it is unlikely that the observed effect is mediated by 1A receptors. Thus, it appears that 1B rather than 1A receptors play a role in these relatively weak suppressant effects on spontaneous XII nerve activity. (Supported by HL47600 and HL42236).

737.3

A STATISTICAL DESCRIPTION OF THE FIRING PATTERNS OF PHRENIC MOTONEURONS. W.-X. Huang*, M.I. Cohen, and Q. He. Dept. of Physiol., Albert Einstein Col. Med., Bronx, NY 10461.

To describe the discharge patterns of phrenic motoneurons (PMNs) statistically, we studied the relation between the interspike interval (ISI) and the order of firing during the inspiratory (I) phase for discharges of 151 PMNs in 8 unanesthetized, decerebrate and vagotomized cats. PMNs were classified as early (n = 62) and late (n = 89) based on the onset delay (border line 20 ms). Statistical analysis showed that for all late-I and 38/62 early-I PMNs, the second ISI in the I phase was significantly shorter than the first. However, for a minority of early-I PMNs (n = 18), all having oscillations that were locked to I onset, the second ISI was longer than the first. Thereafter, for all the PMNs, the ISIs became continually shorter due to gradually increasing drive. The decrease of ISI during the I phase was analyzed with regression and correlation techniques. With the method of least squares, curve-fitting was done to three types of curve: 1) straight line, 2) exponential curve, and 3) power function. We found that, for a given PMN discharge, the power function was the best fit for the relation between ISI and the order of firing in the I phase. This power function had the general form

$$Y = \alpha \cdot (X + c)^{\beta}$$

where Y is the ISI and X is the order of firing in an I phase. This relation held for most PMNs, with a correlation coefficient of 0.996 ± 0.007 (mean ± SD, n = 109), except for the 18 early-I PMNs whose second ISI was longer than the first. This pattern of time dependence of interval decrease may reflect the properties of the medullary inputs and the PMN responses. (Supported by N.I.H. Grant HL-27300.)

737.4

PHRENIC INTERNEURONS DO NOT CONVEY RESPIRATORY DRIVE TO PHRENIC MOTONEURONS. Steve Iscoe* and James Duffin, Department of Physiology, University of Toronto, Toronto, ON, Canada M5S 1A8.

Medullary respiratory neurons may drive phrenic motoneurons directly via monosynaptic connections, indirectly via interneurons, or a combination of the two. We tested the role of phrenic interneurons in transcribing central respiratory drive to phrenic motoneurons by cross-correlating the spontaneous activity of 26 C5 phrenic inspiratory interneurons to that of the ipsi- and contralateral phrenic nerves in decerebrate cats. Ten interneurons discharged only during inspiration (phrenic burst) and 16 tonically with increased firing during inspiration. The cross-correlograms of 21 of the interneurons to the ipsilateral phrenic were flat; 3 cross-correlograms had broad peaks centred about time zero, indicating common activation of the interneurons and motoneurons. The other two had troughs. Of the 22 cross-correlograms to the contralateral phrenic, 20 were flat and 2 had a broad peak at time zero. Despite inspiratory-modulated discharge patterns, phrenic interneurons do not convey central respiratory drive to phrenic motoneurons.
Supported by the Medical Research Council of Canada

737.5

CYCLIC ADENOSINE MONOPHOSPHATE (cAMP) MEDIATES SHORT-TERM MEMORY WITHIN THE PHRENIC MOTOR NUCLEUS. Y. Sun, P.G. Wagner, and M.S. Dekin*. Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903-0019

Within the phrenic motor nucleus (PMN), thyrotropin-releasing hormone (TRH) causes presynaptic facilitation via an increase in cAMP while baclofen, a specific $GABA_B$ agonist, has the opposite effect (Sun *et al., Neurosci. Abstr.*, **20**:544, 1994). In this study we tested the hypothesis that brief exposure to TRH or baclofen would have persistent effects on presynaptic cAMP levels resulting in short-term memory within the PMN. Spinal cord slices containing the PMN (C3 to C5) were obtained from 3 to 7 day old rats. Whole cell recordings were made from motoneurons identified by antidromic stimulation of the ventral root. Excitatory postsynaptic potentials (EPSP) were elicited by stimulating the ventral (or lateral) funiculus. A separate pressure ejection electrode was used to apply TRH (10 μM) or baclofen (100 μM). Pressure ejection of TRH for 30 sec increased the EPSP amplitude while similar application of baclofen reduced the EPSP amplitude. These changes in EPSP amplitude persisted for as long as 1 hour. Motoneuron membrane properties, EPSP kinetics, and responsiveness to exogenous L-glutamate were not altered. Bath application of Rp-cAMP, a competitive inhibitor of cAMP at its binding site on protein kinase A, reduced the EPSP amplitude and antagonized the effects of both TRH and baclofen. These data demonstrate that transient exposure to neuromodulators of cAMP have long lasting effects on neurotransmitter release resulting in short-term memory within the PMN. (Supported by the UMDNJ Foundation, Parker B. Francis Foundation, and NIH Grant HL 02314).

737.6

PROTEIN KINASES A and C MODULATE THE ACTIVITY OF AN OUTWARD RECTIFYING $GABA_B$ ACTIVATED K^+ CHANNEL IN CULTURED PREMOTOR RESPIRATORY NEURONS. P.G. Wagner*, Y. Sun and M.S. Dekin Dept. of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, N.J. 08903.

Baclofen, a $GABA_B$ agonist, activates a Ba^{++}-insensitive outward rectifying K^+ channel [$K_{(OR)}$] in premotor respiratory neurons (Wagner and Dekin, *J. Neurophysiol.* 69:286, 1993). $K_{(OR)}$ channels are inhibited by both cAMP and thyrotropin-releasing hormone (TRH). Both 8-bromo-cAMP and TRH cause an increase in the interval between bursts of channel openings resulting in a dercrease in nPo. These data suggested that cAMP and TRH shared a common mechanism of action.TRH receptors, however, are usually associated with the activation of PKC (Joels and Drummond, *Brit. J. Pharmacol.* 96:450, 1989). In this study, therefore, we compared the ability of antagonists of PKA and PKC to protect $K_{(OR)}$ channels from TRH mediated inhibition. All recordings were made using the patch clamp technique in the cell-attached configuration. $K_{(OR)}$ channels were activated with 100 μM baclofen in the presence of Rp-cAMP (1 μM) or chelerythrine (1 μM). Both Rp-cAMP (n=10) and chelerythrine (n=4) caused a change in the conductance state of the channel from >100 pS (baclofen alone) to < 40 pS and an increase in nPo from <30% (baclofen alone) to >70%. The cells were then exposed to TRH (1 μM) in the presence of baclofen and either Rp-cAMP or chelerythrine. Both antagonists prevented the inhibition caused by TRH. These data suggest that not only does TRH produce its effects via PKC, but that there is an interaction between the PKA and PKC signaling pathways. (Supported by NIH Grant HL02314, the UMDNJ Foundation and Parker B. Francis Foundation).

737.7

SYNAPTIC MODULATION OF PHRENIC MOTONEURON EXCITABILITY IN RATS. D.R. McCrimmon*, F. Hayashi & C. Hinrichsen. Dept. Physiology, Northwestern University Medical School, Chicago, IL 60611-3008.

Modulation of the excitability of phrenic motoneurons was examined in response to stimulation of their descending inputs. Urethane-anesthetized Sprague-Dawley rats were paralyzed and their spinal cords transected at C1-2 to remove spontaneous respiratory drive. Descending pathways were activated using a concentric bipolar electrode in the lateral funiculus just caudal to the transection. Single-pulse stimulation of the descending pathway gave rise to a single, short-latency peak in phrenic nerve activity. In response to paired-pulse stimulation (intervals ≤ 20 msec), the second stimulus elicited two peaks. The first peak was identical to that elicited by the first pulse but was followed at short latency by a second peak, the amplitude of which was dependent upon the interpulse interval. The shorter the stimulus interval, the greater the amplitude of the second peak. Repetitive stimulation (50-100 Hz, 0.2 ms pulse, 3-120 μA, 10-30 sec train) of the descending pathway increased the spontaneous activity on the phrenic nerve for about 1 min. Intracellular recording revealed that the increased excitability arose from a depolarization of individual phrenic motoneurons and an increase in synaptic noise. There was also an increase in the amplitude and duration of individual EPSPs in response to single (test) pulse stimulations of the descending pathway following periods of repetitive stimulation. These data suggest that increases in phrenic motoneuron excitability may contribute to short-term potentiation of breathing. Supported by HL 40336.

737.8

MODELING OF NEURAL MECHANISMS FOR RESPIRATORY PATTERN GENERATION. I. A. Rybak, J. F. R. Paton[1], P. J. Kruk* and J. S. Schwaber. Neural Computation Group, DuPont Central Research, Wilmington DE 19880-0328 [1]Department of Physiology, University of Bristol, Bristol, BS8 1TD, UK

The objectives of the research were: (i) to develop computational models of neural mechanisms that provide the genesis and control of both respiratory oscillations and specific patterns of respiratory neurons, and (ii) to understand the mechanisms of integration and specific roles of intrinsic properties of respiratory neurons, network properties of their interconnections, and effects of afferent feedback in the genesis and control of the respiratory pattern.

Models of the central respiratory rhythm generator (CRG) were developed in the framework of the network theory of respiratory rhythmogenesis. The models of single respiratory neurons were developed in the Hodgkin-Huxley style and include sodium and a series of potassium and calcium channels. The single neuron models produce the specific firing patterns of respiratory neurons recorded experimentally (i.e. adapting and ramping bursts).

Different model versions of the CRG have been considered. They consist of interconnected neurons with different intrinsic properties and peripheral feedback from pulmonary stretch receptors (PSR) and chemoreceptors. The models have the same inspiratory off-switch but different expiratory-off switch mechanisms. They demonstrate both a stable respiratory rhythm and specific patterns of respiratory neuronal discharges. The models are compared and analyzed on the basis of their performance under normal conditions and under the influence of different perturbations applied to the PSR feedback and to afferent inputs from different nerves.

The simulation results indicate the most plausible network architecture and expiratory off-switch mechanism. Our models show how the intrinsic biophysical properties of individual neurons and groups of neurons along with the network properties provide the shaping of the specific respiratory patterns.

737.9

The effect of severe hypoxia on expiratory motor unit activity in the spontaneously breathing cat. J.H. Mateika, E. Essif, D. Fuller and R.F. Fregosi*. Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85721

These experiments examined the effect of steady state severe hypoxia on the discharge frequency of expiratory motor units in anesthesized cats. Expiratory units were localized from the external oblique muscle while the cats expired against a positive end expiratory pressure (PEEP) of 1-2.5 cmH_2O. Subsequent to unit localization, PEEP was removed and unit activity, inspiratory and expiratory airflow, end-tidal oxygen (O_2) and carbon dioxide (CO_2), and external oblique electromyograph (EMG) activity were measured for 3-4 minutes during hyperoxia (F_IO_2 = 1.0) and severe hypoxia (F_IO_2 = 0.08-0.1). Throughout severe hypoxia end-tidal CO_2 was maintained at levels equivalent to that measured during hyperoxia. A total of 15 expiratory motor units were analyzed; 7/15 were active during hyperoxia while 12/15 were active during severe hypoxia. The number of impulses/expiratory period (N/T_E) and discharge frequency (f) of the units increased from a mean (± sd) of 9.5 ± 1.3 and 7.3 ± 3.0 (Hz) during hyperoxia to a mean of 14.1 ± 2.9 and 10.1 ± 3.1 (Hz) during severe hypoxia, respectively. This increase was accompanied by a significant increase in EMG activity (% of EMGmax) from 19.8 ± 18.0 to 48.1 ± 31.3. Cycle triggered histograms of N/T_E and f revealed that during both hyperoxia and severe hypoxia the majority of motor units did not begin to discharge until 30 % of the expiratory phase was completed. In addition, the impulse rate and discharge frequency for a given interval of the expiratory period was always greater during severe hypoxia compared to hyperoxia. Therefore, activation of the external oblique muscle contributes to lung deflation during the last 70% of the expiratory period during severe hypoxia. In addition, both rate coding and recruitment of motor units contribute to the increase in expiratory muscle activity.

737.10

MODULATORY PROCESSES IN THE ISOLATED RESPIRATORY SYSTEM OF NEONATAL AND MATURE MICE. F. Elsen*, U.J.A. Quellmalz, J.M. Ramirez, D.W. Richter. Department of Physiology, University of Göttingen, 37073 Göttingen, FRG.

Transverse medullary slices of mice containing the pre-Bötzinger complex generate spontaneously respiratory rhythmic activity. Inspiratory activity can be recorded at all postnatal stages (P0-31) as mass-activity from hypoglossal (XII) rootlets (Funk et al. 1994, J. Neurophys. 72: 2538; Ramirez et al. 1995, Eur. J. Physiol. 429: 599). Contained within this slice are the anatomical regions C1 and C2 which are characterized by their alpha-2 adrenergic binding sites (Flügge et al. 1990, J. Comp. Neurol. 297: 253). Using the transverse slice as an *in vitro* model, we examined the modulatory action of catecholamines on the isolated respiratory network of the developing mouse. At all postnatal stages (P0-18), blockade of alpha-2 adrenergic receptors by the antagonist yohimbine (1-5 μM) led to a decrease in the frequency of rhythmic activity in XII rootlets and in neurons of the pre-Bötzinger complex, an area which is essential for respiratory rhythm generation (Smith et al. 1991, Science 254: 726). Thus, endogenously released adrenaline seems to modulate central respiratory activity. Consistent with an endogenous adrenergic drive was the finding that adrenaline (5 μM) increased the frequency of XII rhythmic activity in neonatal (P0-5, n=8) and mature mice (P9-18, n=5) by 114 +/-29% and 83.5+/-15%, respectively. The duration of inspiratory XII bursts was increased in neonates (by 47%) and mature mice (by 61%) which correlated with a reduction in the interburst interval. Modulatory changes in XII rootlet activity were also reflected in neurons recorded intracellularly within the pre-Bötzinger complex. Whole-cell patch recordings from inspiratory neurons indicate that amplitude, slope and duration of depolarizating drive potentials were increased in the presence of 5μM adrenaline. Expiratory neurons were depolarized and rhythmic hyperpolarizations enhanced. The data demonstrate that the adrenergic system plays an important role in central respiratory control.

737.11

ELECTROPHYSIOLOGY OF MEDULLARY RAPHE NEURONS IN SLICES AND TISSUE CULTURE. G.B. Richerson* & J.H. Pizzonia, Dept. of Neurology, Yale University & VAMC, West Haven, CT. 06516

Neurons in the medullary raphe project widely to other respiratory nuclei, and contain neurotransmitters which strongly influence breathing. Respiratory acidosis alters the firing rate of pacemaker neurons in this region and in the ventrolateral medulla (Richerson, 1995; J. Neurophysiol. 73(3):933-44), making neurons in both regions candidates for central respiratory chemoreceptors. Changes in CO_2 / pH alter the firing rate of these neurons by modulating pacemaker currents; however, the specific currents responsible for the pacemaker potential are unknown.

In the present work, patch clamp recordings were made from medullary raphe neurons in slices in order to determine which currents were present which could potentially be modulated by respiratory acidosis. Results were compared to data from cultures of microdissected rostral medullary raphe. Neurons had similar properties using both preparations. In current clamp, neurons displayed delayed activation, spike frequency adaptation, short and long duration afterhyperpolarizations, and a voltage sag with maintained hyperpolarization. Whole-cell voltage clamp recording demonstrated a rapid, transient outward current activated by depolarization from potentials negative to resting potential, as well as inward rectification. These findings suggest that currents in neurons of the medullary raphe include A-current, calcium currents, calcium-activated K^+ currents, and an inwardly rectifying K^+ current, each of which can contribute to pacemaker potentials. Any of these currents could be the site of action of pH / CO_2 in modulating repetitive firing in these neurons. Characterization of the electrophysiologic properties of these neurons is essential for determining which ion channels are modulated by respiratory acidosis, and may help define the cellular basis of respiratory chemoreception. (Supported by the VAMC)

737.12

MEDULLARY RAPHE NEURONAL RESPONSES DURING FICTIVE COUGH. R. Shannon*, K.F. Morris, Z. Li, and B.G. Lindsey. Physiol. and Biophy,. Col. Med., Univ. South Florida, Tampa, FL 33612

Raphe neurons influence breathing. Serotonin is known to modulate the cough pattern. This study was motivated by the possibility that raphe neurons may modulate airway sensory information and the respiratory motor pattern during coughing. Decerebrated, thoracotomized, paralyzed, phrenic-triggered ventilated cats (8) were used. Extracellular single neuron activity, and phrenic and lumbar neurograms were monitored during fictive cough produced by mechanical stimulation of the intratracheobronchial tree. Up to 6 neurons were recorded simultaneously with a microelectrode array. Neurons were tested for respiratory modulation of firing rate by cycle-triggered histograms and 2 statistical tests, and for functional linkage to phrenic and lumbar motoneurons by spike-triggered averaging. Of 80 neurons, 10 were expiratory and 7 were inspiratory modulated; 63 had no respiratory modulation. Cells in each category showed either increases, decreases or no change in firing rate during fictive cough. The respiratory modulated cells had only small variations in discharge rate during control respiratory cycles. The results provide evidence for changes in the raphe neuronal network consistent with a modulatory role during coughing. (Supported by NIH HL49813)

737.13

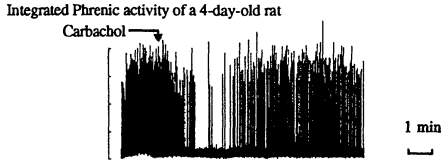
SPATIOTEMPORAL IMPULSE PATTERNS DISTRIBUTED AMONG BRAIN STEM NEURONS RECORDED IN PARALLEL. E. Chang, K. F. Morris*, R. Shannon, and B. G. Lindsey. Dept. Physiol. & Biophysics, Univ. South Florida Med. Ctr., Tampa, FL 33612.

We have reported favored patterns in the spike trains of single neurons that are elements of brain stem cardiorespiratory-related neuronal assemblies identified by their impulse synchrony (FASEB J. 9:A839, 1995). Those results motivated a search for multineuron patterns of impulses that repeat more often than expected by chance. Spike trains were recorded in parallel with electrode arrays in the n. raphe obscurus, n. raphe magnus, and the region of the ventral respiratory group of 9 anesthetized (Dial), vagotomized, artificially ventilated cats. Samples of "spontaneous" activity from 4 to 11 simultaneously recorded single neurons were analyzed with the algorithms of Abeles and Gerstein (J. Neurophysiol. 60:909, 1988). The number of different recurring distributed patterns composed of 4 or 5 spikes exceeded ($p < 0.01$) that expected under the null hypothesis in 12 of 14 data sets. In 6 of 7 data sets, single neurons with previously identified favored patterns were also elements of groups of neurons with "excess" distributed patterns. The results: a) are consistent with the possibility that single neuron patterns may be fragments of distributed impulse sequences, b) provide another line of evidence for the hypothesis that brain stem neurons operate in coordinated assemblies, and c) encourage the search for coding functions of spike patterns in parallel channels. Supported by NS19814.

737.14

RESPIRATORY DEPRESSION BY CARBACHOL INJECTION IN THE PONTINE RETICULAR FORMATION IN NEONATAL RATS. M.-L. Fung* and W.M. St.John. Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756.

In adult animal, injection of cholinergic agonist, carbachol, in the pontine reticular formation decreases phrenic activity (H. Kimura et al., *J. Appl. Physiol.* 69: 2280-9). I postulated that the pontine cholinergic mechanism mediated respiratory depression is present in the neonatal animal. Phrenic activity was recorded in decerebrate, paralyzed, ventilated and vagotomized neonatal rats from 4 to 22 days after birth. Small volume (10-60 nl) of carbachol (44-88 mM) was injected in the medial portion of the rostral pons. The injection of carbachol decreased phrenic peak activity and respiratory frequency in most animals. The site of injection was confirmed by histology. Result suggests that endogenous cholinergic mechanism in the medial pons depresses respiratory activity in neonatal animal. This study provide preliminary data for future studies of the nature of the mechanism underlying respiratory depression in developing animal and the possible link of the mechanism to the sudden infant dead syndrome.



737.15

AXONAL PROJECTIONS FROM THE PONTINE PNEUMOTAXIC REGION TO THE BÖTZINGER COMPLEX IN CATS. M. Aoki*, G. Song, Y. Sato, and I. Kohama. Dept. Physiology, School of Medicine, Sapporo Medical University, Sapporo 060, Japan.

We investigated axonal projections of respiratory and non-respiratory neurons in the pontine parabrachial -Kölliker- Fuse nuclear complex (NPB- KF, the pneumotaxic region) to a distinct group of expiratory neurons, the Bötzinger complex (BOTC), in the medulla by an antidromic mapping technique in chloralose-urethane anesthetized cats. Among 91 respiratory neurons extracellularly recorded in the NPB-KF, 13 neurons (11 inspiratory, 2 phase spannig type) were considered to have axons projecting to the BOTC. Antidromic mappings indicated that those descending axons terminate in the BOTC. Other 55 non-respiratory neurons were also antidromically activated by electrical stimulation of the BOTC. Recording sites of antidromically activated respiratory and non-respiratory neurons were distributed in the lateral NPB and KF nuclei. For histological identification of the projections, a small amount of WGA-HRP (30-50 nl, 5%) was injected into the BOTC. A number of retrogradely labeled cells were observed in the NPB- KF and the distribution of labeled cells was similar to that of recording sites. These results suggested that the pathway from the pontine pneumotaxic region to the BOTC is involved in respiratory control.

737.16

THE KÖLLIKER-FUSE NUCLEUS MEDIATES CARDIO-RESPIRATORY RESPONSES TO TRIGEMINAL NERVE STIMULATION IN THE RAT. M. Dutschmann & H. Herbert*, Dept. Animal Physiol., Univ. Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany.

Stimulations of the nasal mucosa strongly influences the cardiovascular and respiratory outflow. These responses are trigeminally mediated. Recent studies have shown that the parabrachial (PB) and Kölliker-Fuse (KF) nuclei receive prominent somatosensory inputs from the spinal dorsal horn and the spinal trigeminal nuclei. The PB/KF itself has prominent projections to autonomic and respiratory cells groups by which he can strongly modulate their activity. In the present study we investigate whether the PB/KF plays a role in relaying the trigeminally induced cardiovascular and/or respiratory responses. To do so, we stimulated the ethmoidal nerve (EN5) and recorded cardiovascular and respiratory responses before and after injections of the calcium channel blocker $CoCl_2$ into the PB/KF.

Unilateral electrical stimulation of the EN5 resulted in a pronounced depression of respiration (apnoe) and a moderate, but significant bradycardia and pressor response. EN5 stimulations immediately after unilateral injections of $CoCl_2$ into the caudal KF resulted in a significant blockade of the respiratory depression. In contrast, $CoCl_2$ injections into rostrally located parts of the PB/KF showed only weak effects. A recovery of the respiratory response to EN5 stimulation (apnoe) was observed 30 to 60 minutes after the $CoCl_2$ injections. Injections of glutamate into the most effective blocking sites in the KF also caused an apnoe, suggesting that the trigeminal input to the KF is mediated by glutamatergic neurotransmission. The cardiovascular responses were also altered after $CoCl_2$ injections. However, whether these effects are statistically significant has to be evaluated by further experiments.

From our data we conclude, that the KF is an obligatory relay site for the trigeminally induced apnoe. Thus, one biological function of somatosensory inputs to the PB/KF might be the mediation of trigeminal nasopharyngeal (protective) reflexes. (Supported by DFG He 1842/3-2)

737.17

APPLICATION OF *IN SITU* PCR TECHNIQUE REVEALS THE DISTRIBUTION OF AN INWARD RECTIFYING K^+ CHANNEL IN RAT BRAINSTEM. J.S. Lai*, Y. Li, M. Kokhab, & J.L. Feldman. Systems Neurobiology Laboratory, Department of Physiological Science, UCLA, Los Angeles, CA 90095-1527.

We postulated that an inwardly rectifying K^+ channel (IRKC) plays an important role in the modulation of respiratory rhythm *in vitro* (Johnson *et al*, Soc Neurosci Abstr 18: 488, '92). Oligonucleotides identical to the M1 and M2 regions of mouse IRK1 (Kubo *et al*, *Nature* 362: 127, '93) were used as polymerase chain reaction (PCR) primers to amplify cDNA from neonatal brainstem (Lai *et al*, Soc Neurosci Abstr 19: 704, '93). A rat IRKC was detected, cloned, and characterized--rat brainstem K^+ channel 1 (RBSK1) (Lai *et al*, Soc Neurosci Abstr 20: 75, '94). In the present study, our goal is to localize its distribution within the medulla relative to regions affecting respiratory control.

Initial attempts to study the distribution of mRNA for RBSK1 were unsuccessful using conventional *in situ* hybridization techniques, perhaps due to its low copy gene expression. To increase sensitivity of detection, we employed *in situ* PCR, using a modified protocol on paraffin embedded sections of adult rat medulla (Nuovo, G. PCR *In situ* Hybridization: Protocols and Applications. New York: Raven Press, 1992). Supported by NIH Grants HL37941 and NS24742.

737.18

IMAGING OF VENTRAL MEDULLARY SURFACE ACTIVITY DURING TRANSIENT RESPIRATORY EVENTS. G. R. Poe*, D.M. Rector, H.V. Forster, P.J. Ohtake, L.G. Pan, T.F. Lowry, D. Gozal and R. M. Harper. Dept. Anatomy and Cell Biology, UCLA, Los Angeles, CA 90095; Dept. Physiol., Medical College of Wisconsin and Zablocki VA Hospital, Milwaukee, WI 53226.

We examined activity from the ventral medullary surface (VMS) during spontaneous sighs occurring within sleep and waking states in goats, using scattered light imaging procedures. Under sterile surgery, 5 goats were instrumented with electrodes to acquire sleep physiology and diaphragmatic EMG measures, and a miniaturized video CCD camera coupled to a coherent fiber bundle. The optic probe was placed over a site on the rostral VMS which, when cooled, elicited apnea. Following recovery, all-night sleep recordings were taken, and images were collected at 1/s. Sighs, (sustained inspiratory efforts > 2.5 x interval mean) were preceded by a substantial decline in moment-to-moment variability for a period of 4-9 sec prior to onset of the sigh. A significant decline in overall activity accompanied the sigh. We conclude that transient respiratory events such as sighs follow alterations in neural activity within the VMS. *Supported by HL-22418, USPHS-25739 and NIDR DE 07212. G.P. is supported by a Howard Hughes Medical Institute Predoctoral Fellowship.*

737.19

IMAGING OF VENTRAL MEDULLARY SURFACE ACTIVITY CHANGES WITH SPONTANEOUS DIAPHRAGMATIC ACTIVITY. R.C. Frysinger*, G.R. Poe, D.M. Rector, H.V. Forster, P.J. Ohtake, L.G. Pan, T.F. Lowry, D. Gozal and R. M. Harper. Department of Anatomy and Cell Biology, UCLA, Los Angeles, CA 90095; Department of Physiology, Medical College of Wisconsin and Zablocki VA Hospital, Milwaukee, WI 53226.

We examined activity from the ventral medullary surface (VMS) during spontaneous breathing occurring within sleep and waking states in goats using scattered light imaging procedures. Under sterile surgery, 5 goats were instrumented with electrodes to acquire diaphragmatic EMG and sleep physiology measures, and with a miniaturized video camera and coherent fiber optic probe placed over a site on the rostral VMS which, when cooled, elicited apnea. Following recovery, all-night sleep recordings were taken, images were collected at 1/sec, and diaphragmatic activity was integrated over a long time constant. Slow variation in overall diaphragmatic activity was cross correlated with light reflectance. Relatively synchronous correlations occurred for variance with a period of less than 10 sec. This lag calculation varied considerably across sleep-waking states, and sometimes disappeared entirely during slow-wave sleep. This finding suggests variable links between respiratory effort and the VMS, and underscores the importance of state in the central control of respiration. *Supported by HL-22418, USPHS 25739 and NIDR DE 07212. G.P. is supported by a Howard Hughes Medical Institute predoctoral fellowship.*

RESPIRATORY REGULATION: CENTRAL CHEMORECEPTION

738.1

POWER SPECTRA OF GASP-LIKE ACTIVITY IN THE PHRENIC NEUROGRAM ELICITED BY CHEMICAL ACTIVATION OF NEURONS IN THE PRE-BÖTZINGER REGION. M. Akay, I.C. Solomon, N.H. Edelman, and J.A. Neubauer*. Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903.

Progressive brain hypoxia results in a shift from respiratory depression to gasping in peripherally chemodenervated animals. In addition, there is a shift in the autoregressive (AR) spectra of the phrenic neurogram such that peaks are seen only in the 0-30 Hz and 60-120 Hz ranges. Recently, we demonstrated that a gasp-like pattern in the phrenic neurogram can be evoked by chemical activation of neurons in the pre-Bötzinger (pre-Böt) region. To determine whether the power spectra of such gasp-like activity is similar to that seen during hypoxia-induced gasping, we examined the AR spectra of chemical-induced gasp-like activity. Gasp-like activity was evoked by microinjecton of either DL-homocysteic acid (DLH; 10 mM; 21 nl; n=5) or sodium cyanide (NaCN; 1 mM; 21 nl; n=3) into the pre-Böt region in chloralose-anesthetized, vagotomized, and paralyzed cats. We also examined the AR spectra of the eupneic pattern immediately prior to microinjection of DLH or NaCN. The AR spectra of the eupneic pattern consisted of a discrete peak in the 30-60 Hz range and another in the 60-120 Hz range. During chemical-induced gasp-like activity there was a downward shift of the 30-60 Hz peak to frequencies < 30 Hz. Prior to cessation of the chemical-induced gasp-like activity, the low frequency power (< 30 Hz) began to shift back towards the eupneic pattern (30-60 Hz range). These findings demonstrate that the changes seen in AR spectra during chemical-induced gasp-like activity in the phrenic neurogram are similar to those seen during hypoxia-induced gasping. Supported by HL16022, HL07467, HL44678, AHA/NJ 93-G-37.

738.2

INTRINSIC EFFECTS ON MEMBRANE POTENTIAL AND INPUT RESISTANCE OF CHEMICAL HYPOXIA ON CULTURED NEURONS FROM THE ROSTRAL VENTRAL LATERAL MEDULLA (RVLM). E. Mazza*, N.H. Edelman and J.A. Neubauer. Department of Medicine, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ 08903.

In the face of a hypoxic insult, central neurons will decrease activity as a means of conserving energy and avoiding cell death. Recent work *in vivo* has also demonstrated subpopulations of neurons that are excited by hypoxia. The goal of this study was to determine the intrinsic effects of chemical hypoxia on membrane potential (V_m) and input resitance (R_m) on cultured neurons from the RVLM, a brainstem area containing neurons important to central cardiovascular and respiratory control. The studies were performed on neonatal rat neurons (>3 days old) plated on astrocyte monolayers using the whole cell perforated patch clamp technique. The cells were perfused in either mock-CSF or mock-CSF containing low Ca^{2+} (0.5 mM) and high Mg^{2+} (4.0 mM) to block synaptic transmission and were equilibrated with 95% O_2/5% CO_2. Sodium cyanide (NaCN, 1-10 mM) was given as a bolus into the perfusion line and the V_m, R_m, and firing frequency (FF) were analyzed in 18 neurons. Of these 18 neurons, 7/18 (39%) were hyperpolarized and decreased FF, while 11/18 (61%) were depolarized and increased FF with NaCN. These responses were unaffected by synaptic blockade. Excitation was associated with a decrease in R_m while depression was associated with an increase in R_m. In all cases responses were repeatable and reversible. Thus, cells cultured from the RVLM show responses that are intrinsic and not synaptically mediated, similar to those demonstrated *in vivo*. These results further suggest the existence of a population of neurons that are excited by hypoxia and that may act as central O_2 sensors. (Supported by HL16022 and HL07467).

738.3

IN VITRO RESPONSES OF PERIAQUEDUCTAL GRAY NEURONS TO HYPOXIA AND HYPERCAPNIA. J.M. Kramer*, P.C. Nolan and T.G. Waldrop. Depts. of Physiology & Biophysics, Kinesiology, Neuroscience Program and College of Medicine, Univ. of Illinois, Urbana, IL 61801.

Previous studies from this laboratory have demonstrated that hypoxia and hypercapnia stimulate neurons in the ventrolateral medulla and in the caudal hypothalamus both *in vivo* and *in vitro*. In addition, we have recently shown that caudal hypothalamic neurons which respond to hypoxia and possess a cardiorespiratory related discharge project to the periaqueductal gray (PAG). The purpose of the present study was to determine if neurons in the PAG are responsive to hypoxic and hypercapnic stimuli in the absence of input from peripheral chemoreceptors and other brain regions. Brain slices (400-500 μm) containing the PAG were obtained from Sprague-Dawley rats and placed in an interface chamber perfused with nutrient medium bubbled with 95% O_2/5% CO_2. Single unit, extracellular responses of PAG neurons to hypoxia (10% O_2/5% CO_2/85% N_2) and to hypercapnia (7% CO_2/93% O_2) delivered for 60-120 sec. and 3 min., respectively, were recorded. Hypoxia altered the discharge rate in 73% of the neurons tested; only 9% of the neurons responded to hypercapnia. Most (85%) of the hypoxia sensitive neurons were stimulated by hypoxia. Perfusion of the slices with a synaptic blockade medium (low Ca^{++}/high Mg^{++}) did not block the excitatory responses to hypoxia. These findings demonstrate that PAG neurons have an inherent response to hypoxia. These results and our prior findings are consistent with a hypothalamic-PAG-medullary pathway involved in the mediation of cardiorespiratory responses to hypoxia. (Supported by NIH grants HL38726 and HL06296).

738.4

ACETAZOLAMIDE-INDUCED ACIDOSIS IN THE CAUDAL HYPOTHALAMUS INCREASES RESPIRATORY FREQUENCY AND HEART RATE. C.Z. O'Connor and T.G. Waldrop*. Depts. of Physiology & Biophysics, Neuroscience Program and College of Medicine, Univ. of Illinois, Urbana, IL 61801.

Prior results from this laboratory have shown that the respiratory response to hypercapnia is modulated by a GABAergic mechanism in the caudal hypothalamus. Moreover, hypercapnia stimulates a population of caudal hypothalamic neurons with cardiorespiratory basal discharge patterns *in vivo* and *in vitro* in the absence of classical synaptic transmission. The purpose of the present investigation was to determine if a hypercapnic/acidotic stimulation isolated to the caudal hypothalamus elicits changes in cardiorespiratory activity. Therefore, respiratory (diaphragmatic EMG activity) and cardiovascular (arterial pressure and heart rate) responses to microinjections of acetazolamide into the caudal hypothalamus of anesthetized, male rats (Sprague-Dawley) were examined. Coates et al. (*J Appl Physiol* 75:5-14, 1993) have shown that acetazolamide, a carbonic anhydrase inhibitor, causes an increase in PCO_2 and an acidosis at the site of injection. Microinjections of acetazolamide ($\bar{x}$=80 nl, 5 $\times 10^{-6}$ M) into many caudal hypothalamic sites produced significant increases in respiratory frequency and heart rate. Microinjection of equivalent volumes of Ringers (pH and osmotically-adjusted) solution into the same hypothalamic sites had no effects upon cardiorespiratory activity. In contrast, large increases in diaphragmatic activity, heart rate and arterial pressure were elicited by microinjection of a GABA antagonist, bicuculline (5 ng/nL). These results indicate that local hypercapnic/acidotic stimulation of caudal hypothalamic neurons stimulates breathing and heart rate. Thus, the caudal hypothalamus appears to be a site of central chemoreception involved in cardiorespiratory control. (Supported by NIH HL38726).

738.5

EXPOSURE TO HYPERCAPNIC STRESS INDUCES C-FOS EXPRESSION IN THE BRAINSTEM CATECHOLAMINERGIC NEURONS. N.S. Cherniack*, K. Yung, B. Erokwu, and M.A. Haxhiu. Department of Medicine, Case Western Reserve University, Cleveland, Ohio 44106 (USA).

The response of catecholaminergic cells to hypercapnic stress was examined using c-fos as a tracing marker. Exposure of unanesthetized rats (n=8) to 15% $C0_2$ for 60 min induced activation of the c-fos gene, expressed as fos like immunoreactive protein (fos), within brainstem, mesencephalon, diencephalon, and cortical regions. Colocalization studies of tyrosine hydroxylase (TH) and fos protein revealed that the majority of the brainstem TH-containing cells expressed fos immunoreactivity. Numerous double labeled neurons were found in the ventrolateral medullary reticular formation (A1/C1 cell groups), in the dorsomedial and ventromedial subnuclei of nucleus tractus solitarius (A2/C2). TH-containing neurons in the ventrolateral pons (A5 cell group), and in the locus coeruleus also expressed fos like immunoreactivity in response to hypercapnic loading. These results suggest that catecholamine-containing neurons are part of the neuronal network involved in the response to $C0_2$. Supported by HL-25830 and HL-50527.

738.6

LOCALIZATION OF C-FOS IN RESPONSE TO ACIDIC STIMULATION OF THE ROSTRAL VENTROLATERAL MEDULLA. S.D. James, C.O. Trouth*, R.M. Douglas, R.W. Durant, and J.S. Allard. Department of Physiology and Biophysics, Howard University College of Medicine, Washington, D. C. 20059.

The rostral ventrolateral medulla(RVLM) has been implicated in the central CO_2 chemosensory regulation of cardiovascular and respiratory function. In this investigation, the effect of acidic mock cerebrospinal fluid at the RVLM was evaluated for c-fos immunoreactivity. c-fos was found in small interneurons embedded in the marginal glia(MG) of the caudal VLM and RVLM. c-fos-positive cells were also detected in nucleus retroambigualis, lateral reticular nucleus, the area postrema, cranial nerve nuclei X and XII and the n. tractus solitarius of the **caudal medulla. Rostrally**, positively stained cells were noted in the nucleus of the trapezoid body, medial parabrachial nucleus,olivary nuclei, cerebellar nuclei, pontine nucleus, the reticulotegmental nucleus of the pons, and the interpeduncular, red and oculomotor nuclei of the midbrain. These studies demonstrate that acidic mock CSF applied to the RVLM induces c-fos in several respiratory related nuclei and indicates that multiple sites of respiratory chemosensitivity may exist within the brainstem.

Supported by ONR Grant Nr. N0014-94-1-0523.

738.7

CYTOCHROME OXIDASE ACTIVITY AT THE VENTRAL BRAINSTEM ASSOCIATED WITH HYPOXIA. S.M. Johnson, M.E. Reynolds, C.O. Trouth, and R.M. Millis*. Dept. of Physiology & Biophysics, Howard Univ. College of Medicine, Washington, D.C. 20059.

Intramitochondrial cytochrome oxidase activity (CCO) has been used as a marker for hypoxia in myocytes and central neurons. Neurons involved in cardiorespiratory regulation at the caudal ventrolateral medulla (CVLM) respond to hypoxia. The present studies quantified differences in CVLM CCO levels in neurons of rats with a.) ligation of carotid of basilar arteries (10-60 min), b.) topically applied mock CSF (mCSF) 5 min at pH 7.40 or c.) KCN (5 min) control. Incubation of frozen sections was done for 0.5-2.0 h with DAB and cytochrome *c*; and computer analysis of DAB reaction product was calibrated in optical density units (ODU) to a colorimetric standard optimized at 2-h incubation (NIH Image Program). Topical mCSF and carotid or basilar ligation produced CCO levels (.38 ± .01 ODU to .49 ± .02 ODU) that was decreased to .18 ± .01 ODU in apneic (KCN induced; 3-5 s) rats and decreased further to .05 ± .01 ODU in controls. CCO in CVLM neurons appears to decline during hypoxia associated with apnea.

(Support: ONR Grant No. N00014-94-1-0523).

738.8

CYANIDE EFFECTS ON CYTOCHROME OXIDASE ACTIVITY AT THE VENTRAL BRAINSTEM. M.E. Reynolds, S.M. Johnson, C.O. Trouth R.M. Millis & J.A. Holloway*. Dept. of Physiol. & Biophys., Howard Univ. Coll. of Medicine, Washington, D.C. 20059.

Hypoxic responses of cardiorespiratory control neurons at the ventrolateral medulla (VLM) have been described. After a 5-min topical application of KCN (20 mg/mL) to chloralose-urethane anesthetized rats, cytochrome oxidase activity (CCO) was quantified by DAB histochemistry in 20 μm frozen coronal caudal VLM (CVLM) tissue sections. A timed study showed that 2-h incubation was optimal for densitometric analysis of the DAB reaction product (NIH Image Program), calibrated to a colorimetric step-tablet that transforms pixel values from linear light transmittance to optical density units (ODU) or concentration. Control CCO was found in CVLM sections exposed to KCN-free mock CSF (mCSF) and was compared to histochemical (HC) controls incubated for 2 h in 0.7 mg/mL KCN. At the CVLM, KCN produced 3-5 s of apnea. KCN decreased CVLM CCO from .38 ± .01 ODU in mCSF controls to .12 ± .01 ODU and to .05 ± .01 ODU in HC controls. CCO appears to be a marker for the effects of hypoxia and cyanide at the CVLM.

(Supported by ONR Grant No. N00014-94-1-0523).

738.9

A TECHNIQUE TO MEASURE pH_i IN NEURONS OF BRAINSTEM SLICES. N.A. Ritucci, J.S. Erlichman*, J.B. Dean and R.W. Putnam. Department of Physiology & Biophysics, Wright State University School of Medicine, Dayton, OH 45435.

Certain chemosensitive neurons in dorsal and ventral regions of the medulla respond to acid/base changes in the brainstem and are thought to be involved in central chemoreception. **We have developed a technique to measure pH_i of neurons in brain slices using a fluorescence imaging system.** The medullae of 3-12 day old rat pups were excised and sectioned into 100-150 μm slices (unless otherwise stated, all steps conducted at room temperature in 5% CO_2/HCO_3^- buffer: (in mM) 5 KCl, 124 NaCl, 1.3 $MgSO_4$, 26 $NaHCO_3$, 1.24 KH_2PO_4, 10 glucose, 2.4 $CaCl_2$, pH=7.4). Slices were loaded with 4 μM BCECF-AM at 37°C for 15 minutes and then washed for 10 minutes. Individual slices were placed into a 750 μl chamber on the stage of an inverted microscope and superfused with CO_2/HCO_3^- buffer at 2 ml/min. Dye-loaded slices were excited alternately at 500 and 440 nm. The emitted fluorescence at 530 nm was directed through a GeniSysII image intensifier and an MTI CCD72 camera. The subsequent images were processed by Image1/Fluor software. The ratio of 500 to 440 nm fluorescence was measured and converted to pH by constructing a calibration curve using high K^+/nigericin solutions at pH values between 5.8 and 8.6. Slices labeled with a neuron-specific antibody, neuron-specific enolase, showed staining that correlated with that of BCECF-loaded cells. Slices labeled with a glial-specific antibody, glial fibrillary acidic protein, showed staining that was very different from that of BCECF-loaded cells. This indicates that our pH_i measurements were taken from neurons. Average pH_i values of neurons in the ventral and dorsal medullary regions were 7.38 ± 0.02 (n=85) and 7.32 ± 0.02 (n=110), respectively. **These values are the first *in situ* measurements of pH_i in the dorsal and ventral regions of the medulla.** [Supported by NIH HL 46308 and the WSU BMS program.]

738.10

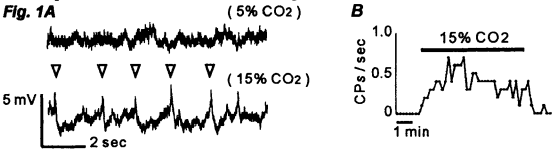
IN VITRO MEASUREMENT OF pHi IN NEURONS IN MEDULLARY CHEMOSENSITIVE AREAS. J.S. Erlichman, N.A. Ritucci, R.W. Putnam, R.-Q. Huang* and J.B. Dean. Department of Physiology and Biophysics, Wright State University, Dayton, OH 45435.

Specific dorsal and ventral sites of the medulla have been identified as chemosensitive, however, the proximate stimulus to these cells is unknown. Previous studies suggest that intracellular pH (pHi) may be the physiologically relevant chemostimulus. We hypothesize that pHi and/or rate of pHi recovery during and after hypercapnia may be unique to cells located within CO_2-chemosensitive areas of the medulla as compared to cells located outside these areas. To this end, we have developed a method for measuring pH_i in neurons in thin (100-150 μm) medullary slices loaded with the pH-sensitive, fluorescent dye, BCECF (see: N.A. Ritucci *et al.*, this volume). Brain slices were prepared from 3-12 day old rats and superfused with a 5% CO_2/HCO_3^- artificial cerebral spinal fluid (ACSF) medium containing (in mM): NaCl 124, KCl 5, $NaHCO_3$ 26, $MgSO_4$ 1.3, $CaCl_2$ 2.4, KH_2PO_4 1.24 and glucose 10 (pH_{ACSF} 7.4). pHi was measured in chemosensitive areas of n. tractus solitarii (avg. ± S.E.; 7.23 ± 0.02, n= 51), dorsal motor n. (7.22 ± 0.09, n= 22) and near the ventrolateral surface (7.44 ± 0.04, n= 41). Non-chemosensitive areas included the spinal trigeminal n. (7.70 ± 0.08, n= 18), *n.* retroambiguus (7.20 ± 0.02, n= 19), inferior olive (7.46 ± 0.03, n= 7), gigantocellular reticular n. (7.20 ± 0.08, n= 7) and ventral reticular n. (7.25 ± 0.03, n= 8). Hypercapnia (10% CO_2) decreased pHi in all cells at all sites to varying degrees (0.77 ± 0.11 pH units, n= 9). Moreover, the rates of recovery of pHi during hypercapnia differed among cells at a given site as well as between sites. We have also found that the ability of cells to regulate pHi during hypercapnic acidosis was critically dependent upon pH_{ACSF}: decreases in pH_{ACSF} of ~0.3 pH units impaired pHi regulation during hypercapnia. [NIH Grant HL 46308, AHA-OH Affiliate Fellowship MV-94-06-F, WSU Research Challenge Award, and the WSU Biomedical Sciences Program]

738.11

ANATOMICALLY COUPLED CO_2/H^+-CHEMOSENSITIVE NEURONS IN THE DORSAL MEDULLA. J.B. Dean, R.-Q. Huang and R.E.W. Fyffe*[,1]. Depts. of Physiol. and Biophysics, and Anatomy[1], Wright State Univ., Dayton, OH 45435.

Morphological properties of CO_2/H^+-excited neurons in the solitary complex (SC; *i.e.*, n. tractus solitarius, dorsal motor n.) were investigated via perforated-patch recordings in transverse, thin slices (~150 µm) prepared from rats (0-14 days old). Chemosensitivity was tested by increasing CO_2 (bal. O_2) in HCO_3^--buffered medium from 5% to 10 or 15% for 5-10 min. The patch was ruptured after establishing the cell's response to CO_2/H^+, thereby permitting lucifer yellow *and* biocytin to stain the cell. Fifteen neurons were anatomically coupled to a second cell; 11/13 coupled neurons were CO_2/H^+-excited. Coupling potentials (CPs ; *Fig. 1A*, ∇) were observed only in anatomically coupled cells, and were maintained during chemical synaptic blockade. CP frequency often increased with high CO_2/H^+ indicating that the adjacent coupled cell was also chemosensitive (*Fig. 1A, B*). CPs were also seen in adult



SC neurons indicating that coupling was not a developmental phenomenon unique to neonates. Our findings demonstrate that anatomical coupling and CPs occur primarily in chemosensitive SC neurons, suggesting that they are electrically coupled via gap junctions. Moreover, CPs are still observed during and after repeated exposures to high CO_2/H^+ suggesting that SC neurons, unlike most other types of cells, are not uncoupled by intracellular acidosis. [NIH Grants HL 46308 and NS 25547]

738.12

ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONS IN CHEMOSENSITIVE AREAS OF THE DORSAL AND VENTRAL BRAINSTEM. T.L. Southard*, R.-Q. Huang, J.B. Dean. Dept. of Physiology and Biophysics, Wright State University, Dayton, OH 45435.

Neurons in the dorsal medullary (i.e., solitary complex, SC) and ventrolateral medullary (VLM) chemosensitive areas are hypothesized to function in central chemoreception. The present study employed the perforated-patch technique (amphotericin B) in thin slices (~150µm; 0-14 day old rats) to characterize electrophysiological responses (e.g., membrane potential, Vm; firing rate; input resistance, R_N) of neurons in SC and VLM to hypercapnia. Individual neurons were visualized using Hoffman Modulation Contrast Optics and exposed for tight-seal recording. Cell sensitivity to hypercapnia was tested by increasing the % CO_2 (bal. O_2) aerating the nutrient medium from 5% (control) to 10% or 15% (hypercapnia) for 5 to 10 min; most experiments were conducted in high Mg^{++}-low Ca^{++} synaptic blockade medium. In the ventral medulla, 28% of neurons tested were CO_2/H^+-excited (i.e., chemosensitive), 32% were CO_2/H^+-inhibited and 40% were CO_2/H^+-insensitive. Twenty-four percent of the neurons in the SC were CO_2/H^+-excited, 29% were CO_2/H^+-inhibited and 47% were CO_2/H^+-insensitive. R_N was either increased or unchanged in CO_2/H^+-excited cells during hypercapnia; R_N changes were variable in CO_2/H^+-inhibited cells and CO_2/H^+-insensitive cells demonstrated no changes in R_N. Depolarizing, excitatory responses to hypercapnia reversed at membrane potentials ~ -90 mV for both SC and VLM chemosensitive neurons. Pretreatment with the potassium channel blocker 4-aminopyridine (4-AP, 2-10 mM) abolished CO_2-induced depolarization. We conclude that CO_2/H^+-excited neurons comprise ~1/4 of the cells tested in the SC and VLM. We hypothesize that CO_2-induced depolarization involves a decreased K^+ conductance based on 1) increased R_N during hypercapnia, 2) reversal of the response at Vm ~ -90 mV, and 3) blockade of CO_2/H^+-excitation by 4-AP (NIH HL 46308, WSU BMS Program).

RESPIRATORY REGULATION: SIGNAL TRANSDUCTION IN THE CAROTID BODY

739.1

ACETYLCHOLINE INCREASES INTRACELLULAR CALCIUM OF CULTURED CAT CAROTID BODY CELLS. M. Shirahata*, J.S.K. Sham, and R.S. Fitzgerald. Departments of Environmental Health Sciences & Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Acetylcholine (ACh) is present in glomus cells of the carotid body (CB). ACh receptors are located on glomus cells as well as carotid sinus nerve (CSN). Exogenous application of ACh to the CB increases CSN neural activity and dopamine release. Perfusion of the CB with blockers of ACh receptors inhibits neural response of the CB to hypoxia. These data suggest ACh may have multiple actions in the CB. As one of the steps to elucidate possible roles of ACh in the CB chemotransduction we examined the effect of ACh on intracellular calcium concentration (Ca_i) of cultured cat CB cells with microfluorometric technique using Indo-1. CB cells were loaded with Indo-1 for 1 hour, and extracellular dye was removed by superfusion of Krebs solution for 45 min. Experiments were performed at 37 °C, and cells were continuously superfused with Krebs solution or Krebs solution containing pharmacological agents. All solutions were equilibrated with 5 % CO_2/16 % O_2. Ca_i was measured from clusters. ACh (1-100 µM) dose-dependently increased Ca_i, and approximately 80 % of clusters tested responded to ACh. Nicotine mimicked ACh effect, and pilocarpine occasionally caused very small increase in Ca_i. Increase in Ca_i by ACh was not blocked by caffeine which depletes Ca from intracellular storage sites. A L-type calcium channel blocker, nifedipine, did not block the effect of ACh. The results suggest that ACh increases Ca_i of cultured CB cells through activation of nicotinic channels. Supported by HL 47044, HL 50712, and HL 52652.

739.2

HYPOXIA DEPOLARIZES CULTURED CAT GLOMUS CELLS BUT DOES NOT INCREASE INTRACELLULAR CALCIUM. Chung-Long Chou*, J.S.K. Sham, Y. Ishizawa, and M. Shirahata. Departments of Environmental Health Sciences & Medicine, The Johns Hopkins Med. Inst., Baltimore, MD 21205

Our previous data using *in vivo* adult cats indicate involvement of voltage sensitive calcium channels (VSCC) for the CB response to hypoxia. We have been culturing CB cells from adult cats, and the cultured glomus cells released dopamine in response to hypoxia. These data are well fit to a current hypothesis of carotid body (CB) chemotransduction. That is, hypoxia depolarizes glomus cells followed by an activation of VSCC. Ca influx through activated VSCC increases intracellular calcium (Ca_i) of glomus cells and triggers neurotransmitter release. We tested the following possibilities if the cat glomus cells are depolarized during hypoxia, and if Ca_i increases during hypoxia. The CB cells cultured on glass coverslips were superfused with Krebs solution equilibrated with 5 % CO_2/21 % O_2, 5 % CO_2/16 % O_2, or 5 % CO_2/0 % O_2. PO_2 in the recording chamber was monitored continuously. Membrane potentials (E_M) of glomus cells were measured with patch clamp techniques. E_M changed from -54±3 mV during hyperoxia to -26±5 mV during hypoxia (20-30 torr; n=4). Sheath cells and other non-glomus cells in the cluster were not significantly depolarized during hypoxia. Ca_i of clusters was measured with microfluorometric technique using Indo-1, and it did not show significant changes during hypoxia. On the other hand, application of 100 mM K^+ clearly increased Ca_i, suggesting the presence of functioning VSCC. Data suggest that under these experimental conditions depolarization of glomus cells during hypoxia may not enough to fully activate VSCC. Supported by HL 47044, HL 50712, and HL 52652.

739.3

CATECHOLAMINE RELEASE FROM RAT CAROTID BODY CULTURES REARED IN NORMOXIC AND HYPOXIC ENVIRONMENTS. A. Jackson and C.A. Nurse*. Dept. of Biology, McMaster University, Hamilton, Ontario, Canada, L8S 4K1.

Chronic hypoxia *in vivo* produces adaptive changes in the chemosensory carotid body (CB) resulting in time-dependent modifications in respiratory functions. Several adaptations to hypoxia have been identified in CB O_2-chemoreceptors (glomus cells) including cell hypertrophy, changes in ion channel expression and neurotransmitter function. By exposing cultures of dissociated Wistar rat CB to different O_2 tensions, we have uncovered some plastic properties of glomus cells that are likely due to direct effects of hypoxia (*P.N.A.S.* 89: 9469, *J. Neurobiol.* 26: 485, *J. Neurosci.* 15: 2192). In this study we begin an investigation of the neurotransmitter status of these cultures by measuring dopamine (DA) release using HPLC (with electrochemical detection). Both acute hypoxia (6 or 10% O_2) and high K^+ medium stimulated DA release from control cultures grown in normoxia for 1-2 weeks. Compared to controls, basal DA release was consistently higher in chronically hypoxic cultures. Further, K^+-evoked DA release (pmol/1000 glomus cells) was significantly higher in chronically hypoxic compared to control cultures after 2 weeks *in vitro*. We are investigating whether these modifications in neurotransmitter function can be correlated with other known physiological and biochemical changes that have been identified in these chemoreceptors after chronic hypoxia. Supported by MRC Canada and the Heart and Stroke Foundation of Ontario.

739.4

CARBONIC ANHYDRASE AND CO_2 CHEMORECEPTION IN FROG OLFACTORY RECEPTOR NEURONS. E.L. Coates* and R.P. Smith. Department of Biology, Allegheny College, Meadville, PA 16335.

There is substantial evidence that the enzyme Carbonic Anhydrase (CA) plays an important role in the detection of CO_2 by central (Coates, Li, and Nattie, 1991; Hanson, Nye, and Torrance, 1981; Erlichman, Coates, and Leiter, 1994) and carotid body chemoreceptors (Lahiri et al., 1993). The objective of the present study was to determine whether the olfactory CO_2 neurons in bullfrogs also use CA to detect changes in CO_2.

To test this hypothesis, olfactory generator potentials were recorded from the ventral epithelium of bullfrogs using glass electrodes (tip diameter = 15-25 µm). Olfactory receptor neurons were tested for their response to 2 second pulses of 5% CO_2 and amyl acetate before and after (up to 90 min) topical CA inhibition with acetazolamide (10^{-2}M) or before and after application of an inactive acetazolamide analog (CL 13850).

In 52 bullfrogs, 1222 sites on the ventral epithelium were checked resulting in 23 locations that exhibited a response to CO_2. It was found that CA inhibition caused an immediate 65% reduction in the generator potential amplitude in response to CO_2 while the response to amyl acetate was not affected. Application of the inactive acetazolamide analog had no affect on the receptor response to either CO_2 or amyl acetate. These results indicate that CA plays a role in the detection of CO_2 by frog olfactory receptor neurons but not in the detection of "normal" odorants and that CO_2 receptors are relatively rare in the olfactory epithelium. The sparse distribution of CO_2-sensitive olfactory neurons was confirmed by histochemical localization of CA in 20 µm cryostat sections, which showed that relatively few olfactory neurons in the bullfrog contain CA. (Supported by NSF grant # IBN 94-09236)

739.5

THE ROLE OF PROTEIN PHOSPHORYLATION IN THE REGULATION OF INTRACELLULAR Ca^{2+} LEVELS AND CATECHOLAMINE (CA) RELEASE IN CULTURED RABBIT CAROTID BODY TYPE I CELLS. L. He, J. Chen*, B. Dinger and S. Fidone. Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT 84108.

Although previous studies have demonstrated that Ca^{2+} currents rapidly degenerate (rundown) in chemosensory type I cells during patch-clamp experiments, it has nonetheless been suggested that Ca^{2+}-channel function in these cells is not regulated by cyclic AMP-initiated protein phosphorylation (*J. Neurochem.* 57: 1992-2000, 1991). In the present study, the presence of MgATP and the catalytic subunit of protein kinase A (PKAc) in patch pipettes markedly slowed the rundown and significantly enhanced the peak values of sustained inward (L-type) Ca^{2+} currents recorded in freshly dissociated chemosensory cells, an effect which was mimicked by okadaic acid, a protein phosphatase inhibitor. Ratiometric imaging revealed that agents which increase cAMP in carotid body type I cells (forskolin, 10 μM; adenosine, 100 μM) also potentiate hypoxia-evoked intracellular Ca^{2+} responses. Finally, a sulfur substituted cell permeant PKA inhibitor, Rp-cAMPS, depresses ^{3}H-catecholamine (synthesized from ^{3}H-tyrosine) release evoked from superfused carotid bodies. The current results are consistent with the hypothesis that hypoxia, which elevates cAMP in type I cells, initiates a cascade of events resulting in elevated intracellular Ca^{2+} levels and enhanced CA release. Supported by USPHS grants NS12636 and NS07938.

739.6

DIFFERENTIAL STIMULUS TRANSDUCTION PATHWAYS IN ARTERIAL CHEMORECEPTOR ORGANS. J. Chen, B. Dinger* and S. Fidone. Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT 84108

In the present study calcium- and calmodulin-dependent protein phosphorylation was evaluated in tissue homogenates of rabbit carotid bodies previously treated with either nicotine (100 μM) or hypoxia (10% O_2 media) *in vitro* (10 min). Initial experiments established the relationship between ^{32}P incorporation (from ^{32}P-γ-ATP) in trichloroacetic acid protein precipitates and varying amounts of Ca^{2+} and calmodulin. Using optimum concentrations of these reagents (0.5 mM $CaCl_2$; 26 μg/ml calmodulin), we subsequently assessed ^{32}P incorporation in the presence or absence of Ca^{2+}/calmodulin as an indicator of calcium/calmodulin protein kinase II (CaMKII) activity. The data show that compared to intact carotid bodies superfused in 100% O_2, prior stimulation with 10% O_2 media did not affect ^{32}P incorporation. In contrast, exposure to nicotine significantly reduced ($p<0.001$) the Ca^{2+}/calmodulin-dependent ^{32}P incorporation. Because these assays were conducted in tissue homogenates following stimulation of intact carotid bodies, the data are consistent with the notion that nicotine, but not hypoxia, had previously activated CaMKII thus occupying many target phosphorylation sites with unlabeled phosphorus groups from the endogenous pool of ATP. Our findings support the hypothesis that nicotine vs. hypoxia activate fundamentally different signal transduction pathways in rabbit chemosensory type I cells. Supported by USPHS grants NS12636 and NS07938.

RESPIRATORY REGULATION: DEVELOPMENTAL MECHANISMS

740.1

DEVELOPMENTAL CHANGES IN THE ISOLATED RESPIRATORY SYSTEM OF MICE: AN *IN VITRO* STUDY USING THE TRANSVERSE RHYTHMIC SLICE. J.M. Ramirez*, U.J.A. Quellmalz, P. Telgkamp and D.W. Richter, Department of Physiology, University of Göttingen, 37073 Göttingen, FRG.

The transverse brainstem slice containing the pre-Bötzinger complex (pBC) has recently been introduced as an *in vitro* system to study the postnatal development of the respiratory system in mice (Funk et al. 1994, J. Neurophys. 72: 2538; Ramirez et al. 1995, Eur. J. Phys. 429: 599). This medullary slice generates at all examined postnatal stages (P0-31) spontaneous rhythmic activity that can be recorded as mass activity from hypoglossal (XII) rootlets. XII rootlet activity is in phase with inspiration and was used to determine phase-relationships of simultaneously recorded rhythmic neurons in the pBC, an area critical for respiratory rhythm generation.

During the first 3 postnatal weeks the duration of XII bursts remained unaltered. However, the pattern of XII bursts changed from decrementing (P1-7) to bell-shaped (P8-22), correlated with a significant decrease ($p=0.008$) in the rise of integrated bursts (-43.3 %). The degree of coupling between bursts in the pBC and those in XII rootlets changed during postnatal development, being 1:1 in a neonatal and on average 3:1 in a mature mouse. No obvious developmental change occurred in rhythm generating mechanisms. At all examined postnatal stages (P0-31) rhythmic activity was abolished by 10μM of the non-NMDA antagonist CNQX. Rhythmicity was maintained in the presence of glycinergic (strychnine, 0.2-20μM) and GABAergic (bicuculline up to 200μM) antagonists. Although inhibitory synaptic mechanisms seem not essential for *rhythm generation*, they are important for *pattern generation*. In XII rootlets blockade of inhibitory mechanisms caused an increase in frequency and burst amplitude and a decrease in burst duration. In the pBC blockade of inhibitory synapses increased rhythmic drive potentials and intraburst spike frequency of inspiratory neurons. The phasic hyperpolarization generated in expiratory neurons was decreased in the presence of strychnine. Following blockade of both glycinergic and GABAergic inhibition expiratory neurons were found to discharge in phase with inspiration.

740.2

DIFFERENTIAL EFFECT OF DOXAPRAM ON THE ISOLATED RESPIRATORY NETWORK OF NEONATAL AND MATURE MICE. B. Wilken*, J.M. Ramirez, U.J.A. Quellmalz, F. Hanefeld, D.W. Richter; Depts. of Physiology and Pediatric Neurology, University of Göttingen, 37073 Göttingen, FRG.

Doxapram is often used in the treatment of idiopathic central apnea in premature infants. It is effective in some cases which do not respond to aminophylline, although in other cases neither of these drugs is successful in treating apnea. These inconsistent effects may be due to maturational changes in the responsiveness of the respiratory system (Peliowski and Finer, 1990 J Pediatr 116:648-653). At present the site of doxapram action and possible developmental dependency remain uncertain. Thus, we examined at different postnatal stages (P0-16) the effect of doxapram on the isolated respiratory network using the transverse rhythmic brainstem slice preparation of mice (Ramirez et al., 1995 Eur J Phys 429:599). These slices (650 - 700 μm) contain the pre-Bötzinger complex (pBC), a region critical for respiratory rhythm generation. Spontaneous respiratory rhythmic activity was recorded extracellularly from hypoglossal (XII) rootlets and intracellularly from neurons within the pBC using the whole cell patch technique. In neonates (P0-4) 20 μM doxapram caused a decrease in cycle length of XII bursts (-74.5% +/- 66) without affecting burst amplitude (5% +/- 13, n=4). In contrast, in mature animals (P5-16), there was a significant increase in cycle length of XII bursts and burst amplitude (56% +/-59 and 79% +/-43, n=8), respectively. This developmental change was not abrupt and at an intermediate developmental stage (P5-8) alteration in cycle length was not significant (65%+/-67, n=4). First effects on burst amplitude were seen at postnatal day 6. Alterations in frequency and amplitude of XII rootlet activity were reflected in similar changes in pBC neuronal activity. Doxapram caused a depolarization of inspiratory neurons. We are currently investigating the cellular basis for this effect. Our data suggest that doxapram acts directly on the central neural network for respiration and that its action is dependent on dramatic maturational changes in the developing mouse.

740.3

DEVELOPMENTAL CHANGE IN THE HYPOXIC RESPONSE OF THE *IN VITRO* RESPIRATORY SYSTEM OF MICE. U.J.A. Quellmalz*, J.M. Ramirez, D.W. Richter, Dept. of Physiology, Univ. of Göttingen, 37073 Göttingen, FRG.

The response of the mammalian respiratory system to hypoxia changes dramatically during postnatal development and several different sites within the respiratory system have been proposed to be responsible for this phenomenon. We examined developmental changes in the hypoxic response of the isolated respiratory network using a rhythmically active medullary slice preparation of mice (Funk et al. 1994, J. Neurophys. 72: 2538; Ramirez et al. 1995, Eur. J. Physiol. 429: 599). Respiratory rhythmic activity at all postnatal stages (P0-31) was recorded extracellularly from hypoglossal (XII) rootlets and intracellularly from pre-Bötzinger complex neurons. Hypoxia, maintained for 20 - 30 minutes, was induced by replacing carbogenated artificial CSF with one containing a mixture of 95% N2 and 5% CO2. In slices obtained from neonatal mice (P0-P7, n=15) hypoxia induced a significant decrease in frequency (34.7% +/-5) which never led to a cessation of rhythmic activity. Duration (-2.1% +/-3.5), amplitude (-5.4% +/-6) and rise (+1.5% +/-5) of XII bursts were not significantly affected by hypoxia. In contrast, in mature animals (P7-P14, n=9) hypoxia caused an initial increase in the frequency of XII activity (+73.5% +/-20) which was accompanied by a dramatic increase in burst duration (+45.5% +/-12), amplitude (+85.6% +/-29) and rise (+29% +/-10) of XII bursts. We refer to this initial phase as *augmentation*. Augmentation was followed by *depression* characterized not only by a decrease in frequency (-20.2% +/-9), but also in burst duration (-9.1% +/-8), amplitude (-21.2% +/-17) and rise (-5.5% +/-18). Depression led always to abolition of rhythmic XII activity (*central apnea*). Inspiratory neurons in the pre-Bötzinger complex were initially depolarized and subsequently hyperpolarized. Expiratory neurons were only hyperpolarized. Under normoxic conditions, both groups showed complete recovery. Our results indicate that the isolated central network for respiration undergoes dramatic maturational changes in its hypoxic response which can now be analyzed under *in vitro* conditions.

740.4

GLOMUS CELL EXCITABILITY AND CAROTID BODY REFLEXES IN NEONATAL AND ADULT RATS ACCLIMATIZED TO CHRONIC HYPOXIA. S.C. Hempleman, F.L. Powell*, and S.M. Asgedom. Dept. of Medicine, University of California, San Diego, La Jolla, CA, 92093.

Chronic hypoxia (CHX) for several days to weeks increases the acute hypoxic ventilatory response (HVR) in adult rats, but has variable effects in neonatal rats. To investigate, we studied HVR and carotid body glomus cell Na^+ and K^+ currents in chronically normoxic (CON, PO_2=140 mmHg) and chronically hypoxic (CHX, PO_2=80 mmHg) adult (24-30 wks old) and neonatal rats (1 week old). Rats were acclimatized to CON or CHX for 3-4 weeks (CHX neonates were gestated and born in hypoxia). HVR was measured by body plethysmography; voltage-gated Na^+ and K^+ currents were measured in dissociated carotid body glomus cells using whole cell patch clamp. In neonates, CHX blunted the acute HVR (10% O_2 for < 1 min; $p<0.05$). Blunting persisted when hypocapnia was prevented with 4% CO_2 in 10% O_2. This contrasts with adult rats in which CHX significantly increases the acute isocapnic HVR. In neonatal glomus cells, CHX decreased I_K density (pA/pF, $p<0.05$), and increased I_{Na} (pA, $p<0.05$). In adult rat glomus cells CHX also decreased I_K density ($p<0.05$), and tended to increase I_{Na}. These results are consistent with increased glomus cell excitability in both adults and neonates after CHX, and suggest that blunting of the HVR in neonates is a CNS effect of CHX. Supported by NIH-HL17731.

740.5

CCK8-SULFATED ALTERS THE TIMING AND PATTERN OF RESPIRATORY BURSTS IN THE NEONATAL RAT *IN VITRO* BRAINSTEM-SPINAL CORD PREPARATION. H. H. ELLENBERGER* and F. M. SMITH, Department of Anatomy and Neurobiology, Dalhousie University, Halifax N.S., Canada, B3H 4H7.

Anatomical evidence suggests a role for cholecystokinin (CCK) in respiratory control (Ellenberger et al., J. Chem. Neuro., 5:375, 1992.). CCK receptors can directly affect neuronal membrane conductances or can act through a variety of second messenger systems. We examined the role of CCK-A and -B receptors on respiratory pattern in the *in vitro* neonatal rat brainstem-spinal cord. Long Evans rats (0-3 day old) (n=12) were anesthetized with ether and the caudal brainstem and cervical spinal cord were removed *en bloc* and placed in flowing artificial CSF solution (pH 7.4, 27°C). Respiratory burst activity was recorded extracellularly from C5 ventral roots. Activation of CCK-A receptors by bath application of CCK8-Sulfated (CCK8-S) doubled respiratory burst frequency at a concentration of 30 and 60 nM. At a CCK8-S concentration of 120 nM, burst duration became prolonged with long augmenting and decrementing phases. There was little or no change in burst amplitude or tonic activity. Bath application of similar volumes and concentrations of the CCK-B agonist, CCK8-Unsulfated, did not produce these effects. The CCK-A specific antagonist, lorglumide (600 nM) blunted or abolished responses to all concentrations of CCK8-S. These results suggest that CCK-A receptors can alter rate and pattern of respiratory burst discharge by acting primarily within the medullary respiratory network and not directly on respiratory motoneurons.

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740.6

PERIODICITY OF SPONTANEOUS RESPIRATORY BURST ACTIVITIES IN NEONATAL RAT MEDULLARY SLICE. Y. Nakazono*, A.Watanabe, H.Shimizu, and M.Aoki, Dept. of Physiol., Sapporo Medical Univ. Sch. of Med., Sapporo 060, Japan

To investigate the neuronal mechanisms of spontaneous respiratory rhythm generation in the brainstem, we analyzed the periodicity of burst intervals in brainstem slice preparations of neonatal rats (1 - 7 days). Transverse slices (750 μm thick rostral to the obex, pre-obex region) were prepared. Spontaneous burst discharges were extra-cellularly recorded from the hypoglossal nucleus (XII n.) and/or the ventrolateral region including the pre-Bötzinger complex. The onset time of each burst was detected with a Schmitt trigger circuit. During the recording (20 - 60 min), burst to burst intervals (T) were automatically determined by means of a digital clock counter. The bursting rhythm of XII n. steadily continued for a few hours in the seven days after birth preparation, with the mean interval of 7.52 s and coefficient of variance of 0.16. We compared these statistical rhythm properties between of medullary neuronal discharges and of C5 rootlet nerve discharges obtained from the brainstem and spinal cord preparation (previously reported). The results showed that the rhythm from a single medullary slice had similar statistical properties to that from the brainstem and spinal cord preparation. It is suggested that the pre-obex region is a sufficient subsystem for generating the respiratory rhythmic bursting in the brainstem and spinal cord in neonatal rats.

740.7

SEROTONERGIC INNERVATION OF MEDULLARY RESPIRATORY CENTERS DURING RAT PRE- AND POSTNATAL DEVELOPMENT. S. M. Wagner and S. W. Schwarzacher*. Department of Clinical Anatomy, Univ. of Göttingen, D-37075.

Serotonin (5-HT) exhibits powerful modulation of central respiratory control during ontogenesis of the mammalian brainstem respiratory network. In addition, it has potential trophic effects on development of synaptic interaction.

We mapped for 5-HT-Immunoreactivity (5-HT-IR) in the ventral respiratory group (VRG) including the pre-Bötzinger-Complex in pre- and postnatal rat brainstems. 5-HT-IR cell bodies of the local caudal raphe nuclei appeared at embryonal day 14 (E 14), terminals with varicosities were detected in the VRG at E15. The number and density of 5-HT-IR fibres as well as fibre branchings within the VRG increased rapidly during the following three weeks reaching adult levels at postnatal day 14 (P 14). In contrast, 5-HT-IR terminal varicosities were densely distributed already at E16-17 and remained at a constant level of density during maturation. Therefore, local serotonergic innervation appears to be present during the entire period of pre- and postnatal development of medullary respiratory centers. The increase in 5-HT-IR fibre density may reflect the development of synaptic interaction within the respiratory network. *Supported by DFG*

740.8

SPATIOTEMPORAL EXPRESSION OF POLYSIALATED NCAM DURING PHRENIC NERVE OUTGROWTH AND INTRADIAPHRAGMATIC BRANCHING. Allan, D.W.*and Greer, J.J., Div. of Neuroscience, U. of Alberta, Edmonton, Alberta T6G 2S2, Canada

During the second and third trimesters of rat gestation, phrenic motoneurons grow out from the cervical spinal cord (C3-C5) through the developing thoracic cavity to innervate the diaphragm in a highly stereospecific manner. Towards understanding some of the molecular events associated with these processes, we examined the pattern of expression of growth associated protein (GAP-43) and polysialyated neural cell adhesion molecule (PSA-NCAM) in the phrenic nerve and diaphragm (E11-P0). Immunohistochemical detection of GAP-43, PSA-NCAM and NCAM was performed using anti-GAP-43 MAb (Sigma), 12E3 MAb (T. Seki) and polyclonal anti-NCAM (E. Bock), respectively.

PSA-NCAM expression was discretely localised along growing phrenic axons at three major directional decision areas: i) at the brachial plexus, where phrenic and brachial axons diverge; ii) at the primary branching site within the diaphragm, where three distinct branches form; iii) along intramuscular branches, where axons separate from the nerve trunk to innervate developing myotubes. The expression of PSA-NCAM in the phrenic nerve thus correlates spatiotemporally with episodes of defasciculation and motoneuron pool-specific guidance. There was also a clear spatiotemporal distribution of PSA-NCAM in developing diaphragmatic myotubes which was correlated with the regional innervation and intramuscular branching of the phrenic nerve and possibly secondary myogenesis. Functional studies of the role of PSA-NCAM were performed by looking at perturbations induced by cleavage of the PSA moiety from the NCAM molecule via intra-amniotic administration of the enzyme endoneuraminidase (U. Rutishauser). Funded by MRC and AHFMR.

740.9

MEDIUM FREQUENCY OSCILLATIONS DOMINATE THE PHRENIC NERVE DISCHARGE OF NEWBORN RATS. R.P. Vertes* and B. Kocsis.
Center for Complex Systems, Florida Atlantic University, Boca Raton, FL, 33431

In adult animals, the discharge of the phrenic nerve, representing central inspiratory activity contains two components termed medium frequency oscillations (MFO), in the range of 20-50 Hz, and high frequency oscillations (HFO), in the range of 50-100 Hz. It was demonstrated in the cat and pig that while MFOs were present in newborn animals, HFOs appeared later in the development and were therefore considered an index of maturation of the respiratory system. It has also been found in kittens that the switch in phrenic nerve activity from MFO to HFO dominance coincided with a characteristic change of the respiratory response to hypoxia from a biphasic response to sustained facilitation of respiration, suggesting that the respiratory system in these species exhibits an abrupt transition from immature to mature pattern in early infancy and that the two measures reflect this transition (Sica & Gandhi, 1990). Although the biphasic respiratory response to hypoxia has been described in the rat, frequency characteristics of the rat phrenic nerve discharge have only been studied in an *in vitro* preparation (Smith et al.,1990).

In this study we examined the synchronization of the discharge of phrenic motoneurons in anesthetized rat pups 14 to 27 days of age. We found that the inspiratory phrenic nerve activity, its first half (ramp) in particular, consisted of synchronized bursts separated by 20-35 ms, corresponding to MFOs. Accordingly, the autospectra of the neurograms had two peaks, one at the respiratory rate and the other at 30-35 Hz. The latter was relatively broad, occupying a range of frequencies from 15 to 50 Hz. These findings demonstrate that phrenic nerve discharge of rat pups, like that of kittens and piglets, is in the MFO range, and suggest that MFO activity is an index of an early developmental stage of the respiratory system.

740.10

SEIZURES AND ELECTROCORTICOGRAPHIC DEPRESSION AFTER RAPID-ONSET HYPOXIA IN YOUNG PIGLETS. K.A. Waters, C. Beardsmore, G.A. Turner, J. Paquette, B. Meehan, I.R. Moss*. Developmental Physiology Lab., McGill Univ., Montreal Children's Hospital, Montreal, PQ, H3H 1P3.

To determine whether the severity or the pattern of hypoxia is important to the induction of seizures, electrocorticographic (ECoG) depression and gasping respiration, cortical electrodes and an arterial cannula were implanted chronically in 16 piglets age 10-22 d. In 48 studies, piglets were exposed to rapid-onset hypoxia of 6%, 8% or 10% on alternate days. Animals were awake, moving freely and hypoxia was either continuous (CH) or repetitive (RH), lasting 21 min in total. RH comprised 7 x 3-min epochs alternating with 3-min recovery epochs in air. ECoG & respiratory responses were filtered and digitally acquired, to permit spectral analysis of the ECoG signals. The ECoG frequency spectrum shifted towards the delta band during hypoxia. Seizures and ECoG depression occurred repeatedly in 6 of 12 piglets exposed to 6% RH, whereas a single event occurred in 2 of 8 piglets during 6% CH. Cortical depression was more marked when hypoxia occurred in brief, repeated cycles as compared to CH of equivalent severity and duration, suggesting particular sensitivity of the cortex to rapid changes in oxygen tension at the onset of hypoxia. Respiratory and metabolic adjustments in CH may aid in the recovery from ECoG events.

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741.1

MATHEMATICAL MODELLING OF PERIODIC BREATHING AT HIGH ALTITUDE: A COMPARATIVE STUDY OF NORMAL ADULTS DURING SLEEP AND AWAKE CONDITIONS. K. Sentrayan[1], A. Thorpe, Jr.[1], A. J. Trouth[2], C.O. Trouth[1,2], S. Rapisardi[3], Depts. of Physio. & Biophys.[1], Neuro.[2], Anatomy[3], Coll. of Med., Howard Univ., Wash., D.C. 20059

A mathematical model has been developed for a comparative study of normal adults during sleep and awake conditions at high altitude. The model considers the controlling effects of both central and peripheral chemoreceptors and has lung, body, and brain tissue compartments. This model also considers time delays and circulatory mixing. We have calculated the peripheral CO_2, O_2, and central CO_2 loop transfer functions using a perturbation approach. Vector addition of the peripheral CO_2, O_2 and central CO_2 loop transfer functions is used to calculate the overall system loop gain (LG) and phase lag (ϕ). The stability and oscillating conditions for periodic breathing of normal adults, during sleep and wakefulness at various altitudes have been compared using the calculated LG and ϕ. The increase in overall system loop gain and decrease in cycle time is observed at high altitudes both in sleeping and awake normal adults. We postulate that during sleep at high altitudes both central and peripheral controllers are affected whereas at sea level, only the central controller is being affected by sleep while the peripheral controller remains unchanged. Supported by ONR Grant No.: N0014-94-1-0523.

741.2

SPINAL SEROTONIN RECEPTORS MODULATE BREATHING PATTERN DURING EXERCISE WITH INCREASED DEAD SPACE IN GOATS. D.L. Turner*#, G.S. Mitchell, K.B. Bach and K.T. Foley. University of Wisconsin, Madison, WI 53706, USA and #University of Leeds, Leeds LS2 9NQ, UK.

Increased dead space (ΔV_D; 0.2-0.3 L) augments the exercise ventilatory response, maintaining arterial PCO_2 regulation from rest to exercise (short term modulation, STM). Thoracic, intrathecal administration of the 5-HT receptor antagonist, methysergide, attenuates the ventilation increase (ΔV_I) and increases $PaCO_2$ during exercise with ΔV_D (*FASEB J.*, 9: A666, 1995). As STM is mediated by an increased tidal volume (ΔV_T), without change in the frequency response (ΔF_R) to exercise, we hypothesized that intrathecal methysergide would attenuate ΔV_T and respiratory drive ($\Delta[V_T/T_I]$) without effect on ΔF_R during exercise with ΔV_D. Goats ran on a treadmill at 4.0-4.8 km/hr, 5% grade, with and without ΔV_D, before and after intrathecal (T_8-T_{10}) methysergide (0.03 mg/kg) or saline. Methysergide significantly attenuated ΔV_I, $\Delta[V_T/T_I]$ and ΔF_R during exercise with ΔV_D (mean±SEM; n=7; *, $p<0.05$), but there was no change in ΔV_T after drug administration. Intrathecal ketanserin (0.3mg/kg, n=4), a more selective 5-HT_2 receptor antagonist, increased $PaCO_2$ during exercise with ΔV_D, indicating a loss of STM, but did not significantly affect ΔF_R.

	ΔV_I	ΔV_T	$\Delta[V_T/T_I]$	ΔF_R
Saline	46±6	0.32±0.07	1.41±0.20	43±6
Methysergide	38±5*	0.29±0.07	1.05±0.15*	34±4*

Thus, during STM: 1) spinal 5-HT receptors modulate respiratory drive (*ie.* V_T/T_I), possibly *via* changes in spinal respiratory neuron excitability mediated by 5-HT_2 receptors; and 2) respiratory timing (F_R) is altered *via* effects on primary afferent transmission, possibly *via* 5-HT_1 receptors in the dorsal horn (NIH HL36780).

741.3

INCREASED SPINAL MONOAMINE CONCENTRATIONS FOLLOWING THORACIC DORSAL RHIZOTOMY (TDR) IN GOATS. G.S. Mitchell*, K.T. Foley, P.A. Martin, E.B. Olson, V. Miletic and M.S. Brownfield. University of Wisconsin, Madison, WI, 53706.

In goats, TDR from T_2 to T_{12} increases spinal serotonin (5-HT) immunoreactivity in rostral thoracic segments (*FASEB J* 6: 1507,1992). To determine if changes in other descending modulatory systems occur following TDR, dopamine (DA), norepinephrine (NE) and 5-HT concentrations were assessed *via* HPLC in the cervical and thoracic spinal cords of unoperated control animals (n=4), following sham surgery (n=3) and 3-12 months following TDR (n=7). Following barbiturate overdose, spinal cords were harvested and frozen for HPLC analysis of homogenized samples. Compared to sham and control goats, TDR increased DA in the cervical (C_5-C_7, 125%), rostral thoracic (T_2-T_7, 190%) and caudal thoracic (T_8-T_{12}, 220%) spinal cord (all $p < 0.0003$). Smaller NE elevations were observed in these regions (28-50%, $p < 0.0006$). 5-HT was elevated 34% between T_2-T_7, but only 16% between T_8-T_{12}; 5-HT concentrations were elevated 68% in the cervical region ($p < 0.002$). The results indicate that TDR increases spinal concentrations of neurotransmitters associated with descending modulation of spinal sensory-motor integration. Since effects were observed in both the denervated thoracic region, and in cervical regions associated with the phrenic motor nucleus, it is possible that changes in monoaminergic brainstem-spinal cord pathways play a role in the functional deficits and/or recovery of ventilatory control that are observed in goats following TDR (NIH HL 36780).

741.4

HYDRALAZINE DECREASES APNEA-INDEX IN SPONTANEOUSLY HYPERTENSIVE RATS. D. W. Carley, S. Trbovic, D. Monti, M. Radulovacki*. Departments of Medicine and Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612

Spontaneous apneic events have been described in normotensive Sprague-Dawley and Wistar rats, and compared to central apneas in man. In view of the epidemiological association between apnea and hypertenison in man, we hypothesized that spontaneously hypertensive (SHR) Wistar-derived rats would exhibit sleep related apnea which could be suppressed acutely by anti-hypertensive agents. We administered 2 mg/Kg of hydralazine to 9 adult male SHR rats implanted with EEG and EMG electrodes for sleep/wake scoring and placed in unrestrained body plethysmographs for respiratory monitoring. For each rat, sleep and respiratory activity were polygraphically recorded for 6 hours on 2 different days. Immediately prior to each recording, saline or drug was given by intraperitoneal injection. We defined apnea as cessation of respiratory effort for at least 2.5 seconds and expressed apnea indexes (AI) as apneas per hour. The effect of hydralazine, documented on a different day by tail-cuff plethysmography, was significant ($p<.0001$) and consistent for 6 hours after injection: baseline = 219.7±4.2, 2Hrs = 157.4±6.0, 4Hrs = 167.0±8.4, 6Hrs = 169.6±7.3 mmHg. During NREM sleep, AI decreased from 12.9±1.4 for saline injection to 5.1±1.3 following hydralazine ($p < .025$). In contrast, during REM sleep, AI did not decrease ($p > .97$); 14.9±3.0 after saline, 14.0±4.1 after hydralazine. The response to hydralazine was not affected by the presence or absence of an augmented breath preceding the apnea ($p > .2$). We conclude that SHR rats exhibit frequent apneas during all stages of sleep. Further, we conclude that acute lowering of mean blood pressure significantly reduces apnea expression in NREM but not in REM sleep. We speculate that baroreflexes may play a state-dependent role in apnea genesis in the rat.

741.5

ROLE OF THE FASTIGIAL NUCLEUS (FN) ON VAGALLY MEDIATED RESPIRATORY RESPONSES IN CATS F. Xu and D.T. Frazier*. Dept. of Physiology, Univ. of Kentucky, Lexington, KY

The FN within the cerebellum has been shown to modify the respiratory responses to hypoxia and hypercapnia (Xu, et al., FASEB, 1995). The goal of this study was to determine the involvement of the FN in the respiratory reflexes evoked by activation of vagal afferents. Experiments were performed in anesthetized (chloralose), paralyzed, and artificially ventilated cats with an occipital exposure of the cerebellum. The phrenic neurogram was used as an index of respiratory motor output. Administration of capsaicin (CAP, 5 μg/kg) via the right external jugular vein and Lung inflation (LI, 10 cmH_2O) were carried out to stimulate non-myelinated and myelinated vagal afferents, respectively. The cardiorespiratory baseline variables and immediate responses (expiratory and inspiratory duration, T_E and T_I; arterial blood pressure, ABP) were compared before and after bilateral lesions (thermal) of the FN. We observed that both stimuli led to a remarkable prolongation of T_E (apnea) and reduction of ABP (with little change in T_I). Bilateral vagotomy abolished all the responses except the decrease in ABP noted during LI. FN lesions did not alter baseline variables and the responses to LI, however, did significantly prolong the response of T_E to administration of CAP. It is concluded that the FN is involved in vagally mediated respiratory responses elicited by activation of non-myelinated vagal afferents. (NIH P01 40369)

741.6

CENTRAL COMMAND INCREASES TOTAL LUNG RESISTANCE IN DECEREBRATE CATS. A.M. Motekaitis, A.C. Bonham*, and M.P. Kaufman. Division of Cardiovascular Medicine, University of California, Davis, CA 95616

The neural mechanisms mediating the airway dilation evoked during exercise are not completely understood. Two mechanisms believed to play important roles in the cardiovascular and respiratory adjustments are the muscle reflex and central command. For example, activation of group III and IV endings in the skeletal muscles reflexly dilates the airways and increases ventilation, heart rate and arterial pressure. Similarly, stimulation of central locomotor regions (central command) increases ventilation, heart rate and arterial pressure. Whether central command plays a role in the control of airway caliber during exercise is not known. Therefore, we tested the hypothesis that stimulation of the mesencephalic locomotor region (MLR) in paralyzed, decerebrate cats decreased total lung resistance (TLR). Electrical stimulation of the MLR increased TLR (27.3±2 to 37.5±3 cm H_2O/l/s, $p<0.05$; n=21). Similarly, microinjection of a GABA antagonist (100-800 nl of 5 ng/nl picrotoxin) into the MLR also increased TLR (24.1±4 to 30.8±6 cm H_2O/l/s, $p<0.05$; n=7). In one site, picrotoxin decreased TLR. All of the changes evoked by electrical and chemical stimulation of the MLR were accompanied by an increase in ventral root discharge and arterial pressure. In 10 cats, the tibial nerve was electrically stimulated at C-fiber intensity, which decreased TLR (30.4±3 to 23.0±2 cm H_2O/l/s, $p<0.05$). Our findings provide little evidence for a role of central command in mediating the airway dilation during exercise. Instead, this dilation may be mediated by peripheral feedback mechanisms, one of which is the muscle reflex. Supported by NIH HL40910.

741.7

REFLEX VOCAL CORDS ACTIVITY ELICITED BY SUPERIOR LARYNGEAL NERVE (SLN) STIMULATION. J. García Ramos*. A. Hernández A. C. Eguía Lis, and L. Hernández A. Neurophysiological Laboratory. Univ. A. de Querétaro and E.S. de Medicina del IPN México D.F. C.P. 11340.
Inhibitory effects upon breathing followin SLN stimulation are widely known, but excitatory effects upon this, or any other structures, have not been described. In pentobarbital anesthetized cats and rabbits observations were made by recording pressure changes in the separated larynx or the mechanical changes obtained from a separated vocal cord, tying a thread at a piece cut from the anterior medial part of the thyroid cartilage and connecting it to a myograph (Model FT 0.3 Grass). The findings were: 1. Single shocks to the SLN 0.1 msec (threshold about IV, maximal 4-5V) elicited phasic laryngeal pressure reductions, 20 msec latencies, or tension increases of the Vocal cord with similar characteristics. Sometimes the responses were followed by changes in the opposite direction thatsuddenly or gradually appeared turning a monophaphasic into a disphasic response. 2. A train, (10-20 Hz) applied for 5-30 sec, produced potentiation of the responses to single shocks lasting for minutes depending on the duration of the train. Similar long lasting potentiation occurred after a long duration pressure pulse to the larynx. It is concluded that afferents in the SLN have excitatory effects upon the premotoneurons of the vocal cords apart from its inhibitory action upon brathing.

741.8

CENTRAL AFFERENT AND EFFERENT PROJECTIONS OF THE TRACHEA - A COMPARATIVE STUDY. M.A. Haxhiu* and A.D. Loewy. Department of Medicine, Case Western Reserve University, Cleveland, OH 44106 (USA)* and Department of Anatomy and Neurobiology, Washington University School of Medicine, St.Louis, MO 63110 (USA).

The central sensory projections arising from the extrathoracic trachea was determined using cholera toxin ß-subunit as a transganglionic tracer in dogs, ferrets and rats. In all three species, tracheal sensory fibers were found to terminate in three subnuclei of the nucleus tractus solitarius (NTS). The most dense concentration of afferent terminals was localized in a restricted part of the medial NTS found at the rostral levels. Less dense projections were identified in the ventrolateral NTS subnucleus and very light projections were seen in the commissural NTS subnucleus. No labeling was identified in the area postrema. In addition, the pattern of retrograde cell body labeling was studied in all three species. The vagal motor supply to the trachea arises from two main sites: the rostralmost part of the dorsal vagal nucleus and the rostral nucleus ambiguus (NA). In the NA, cell labeling was found in both the compact and ventral (or external) portions, with a considerable concentration in the latter. In addition, labeled neurons were consistently seen in the dorsomedial part of the ventral horn in the C1-C2 spinal cord. The present study demonstrates that three different types of mammals share a common pattern of central sensory innervation and motor outflow that is involved in airway control. Supported by HL-50527.

741.9

VESTIBULAR EFFECTS ON THE UPPER AIRWAY.
Alan D. Miller* and Marina S. Siniaia. Lab. Neurophysiology, The Rockefeller University, New York, NY 10021.

Activation of the vestibular nerve produces reflex responses recorded from respiratory muscle nerves of the thorax and abdomen (Yates, Jakus, Miller, *Brain Res.* 629: 209-217, 1993). In order to better understand the functional significance of vestibulo-respiratory reflexes, we investigated the extent to which such responses are also present on respiratory muscle nerves of the upper airway.

Experiments were conducted on adult cats that had a midcollicular decerebration. The vestibular nerves were electrically stimulated at intensities less than those required to produce current spread to the nearest non-target (i.e., facial) nerve. Vestibular-evoked responses were recorded from the following nerves: recurrent laryngeal, superior laryngeal, hypoglossal, glossopharyngeal, and pharyngeal branch of the vagus nerve. The responses could consist of an increase and/or decrease of nerve discharge. Response latencies (from the 1st stimulus of a 5-shock train) were less than 20 ms; response durations were typically between 12-27 ms. Injections of the neurotoxin kainic acid into the medial and inferior vestibular nuclei abolished the responses.

Thus, the widespread presence of vestibular-evoked responses on respiratory muscle nerves of the upper airway, as well as those of the thorax and abdomen, suggests that one function of vestibulo-respiratory reflexes is to provide adjustments in breathing and airway patency during movements and changes in posture. This does not exclude additional possible roles for vestibular-respiratory reflexes, e.g. in assisting venous return to the heart to counter orthostatic hypotension or in maintaining posture. Supported by NIH grants NS20585 and DC02644.

741.10

RELATIONSHIP BETWEEN EXPIRATORY RESISTIVE LOAD MAGNITUDE AND EVOKED POTENTIAL AMPLITUDE IN ADULTS. C. Smith Hammond,* P.W. Davenport. Dept. of Physiol. Sciences, JHMHC, University of Florida, Gainesville, Florida, 32610.

Respiratory related evoked potentials (RREP) recorded from frontal, temporal, parietal and central sites have been elicited by occlusion of expiration. The initial positive peak (P_1) peak observed in the central electrodes is believed to represent the sensory cortical response to the activation of the afferents transducing the expiratory load. An earlier positive peak P_F was observed in frontal sites only. It was hypothesized that the P_1 peak amplitude is proportional to the expiratory load magnitude in adults. Normal adults were studied semi-reclined at rest. They respired through a mouthpiece and non-rebreathing valve connected to a loading manifold. EEG activity was recorded from C_3, C_4, C_Z, C_3', C_4', C_Z', P_3, P_4, P_Z, T_3, T_5, F_Z, F_3, referenced to joined earlobes. The expiratory resistive (R) load magnitudes were 5.0 and 20.0 cm H_2O/L^{-1} and complete occlusion. A control trial obtained by interruption of expiration with the no-load, R_0, port open. The P_1 and P_F peaks of the RREP elicited by expiratory occlusion were measured for peak amplitude and latency. No characteristic peaks were were observed with the control R_0. The P_1 peak was found with both R loads and occlusion. The P_1 and P_F amplitude increased with increasing R load magnitudes. There were no significant differences between P_1 latencies for all loads. The P_F was found consistently in the frontal electrodes and there were no significant differences between P_1 latencies for all loads. These results support the hypothesis that graded increases in expiratory load are correlated with increased activation on sensory cortical neurons. Supported by NIH/NHLBI grant HL 48792.

741.11

A COMPARISON OF CEPHALIC REFERENCED WITH JOINED EARLOBE REFERENCED INSPIRATORY OCCLUSION ELICITED RREP RECORDS. H. Gaeta , C. Smith-Hammond, P.W. Davenport.* Dept. of Physiol. Sci., JHMHC, University of Florida, Gainesville, Florida, 32610.

Respiratory related evoked potentials (RREP) elicited by inspiratory occlusion were initially recorded from C_3 and C_4 with a cephalic reference. Subsequent respiratory related evoked potentials have also been reported using either an earlobe or Spinal C7 reference. The differences in the RREP waveforms raise the question whether changes in reference site alters the characteristics of the signals recorded. This study compares RREP records referenced to joined earlobe with C_z referenced records. Children between 7-15 years were the subjects. Electrodes were applied to C_3 and C_4 and referenced to C_z and joined earlobes. Two trials of 80 inspiratory-interrupted occluded breaths and 1 control trial of 80 unoccluded breaths were presented. Averaged control and occlusion records were compared to determine the presence or absence of the first short-latency positive peak (P1). When P1 was observed, the peak latency and amplitude were determined. Analysis of the results showed that with C_z referencing P1 was observed bilaterally. With joined earlobe referencing P1 was also observed bilaterally. The P1 amplitude with C_z referencing was greater and had a longer latency than with joined earlobe referencing. The observed differences in the RREP P1 peak with the different references can be explained in terms of the increase in noise using a joined earlobe reference. This increases the threshold for resolution of the P1 component from background noise and decreases the amplitude. We conclude that if the reference site is changed when recording RREPs, then consideration must be given to the number of sweeps necessary to maintain a comparable signal to noise ratio between records. (Supported by NIH-NHLBI#HL48792).

742.1

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH)-ASTROGLIA INTERACTIONS: EFFECTS OF ASTROGLIA-DERIVED AND EXOGENOUSLY-DELIVERED GROWTH FACTORS (GFs). E. Gallo[1]*, B. Marchetti[2] and A. Beaudet[2]. [1]Department of Pharmacology, Medical School, University of Catania, 95125 Catania, Italy and [2]Montreal Neurological Institute, 3801 University, Montreal, Quebec, H3A 2B4

In recent studies we have provided experimental support for the concept that a significant degree of communication between neurons and their associated glial cells contributes to the developmental regulation of luteinizing hormone-releasing hormone (LHRH) neuronal function. To further characterize and quantify the morphogenic impact of LHRH neuron-astroglia interactions, we have used the immortalized hypothalamic LHRH GT_{1-1} neuronal cell line and astroglial cells in primary culture. In this experimental design, astroglial cells at different "ages", ranging from 7 to 16 days for "young" glia and 21 to 40 days for "aged" glia, and GT_{1-1} neurons were grown in the same wells, and the analyses carried out at different time intervals (2-8 DIV) during LHRH neuron differentiation. In this paradigm, astrocyte-derived factors (ADFs) induced dramatic effects in the morphology and functional capacity of the GT_{1-1} neurons. First there was an acceleration in the acquisition of the mature neuronal phenotype as evidenced by the extension of multiple lengthy neurites that contact distant cells, or end in apparent growth cones, as well as the establishment of cell-cell contacts. Quantification of the morphometric features of the GT_{1-1} neurons for process length and branching revealed a 3- to 4-fold increase in the number of LHRH processes per cell, as well as a significant increase in the length and branching of individual process, as compared to neurons grown in the absence of glia. Such effects were accompanied by a sharp stimulation of LHRH release in the medium at each time-interval studied. Some of these effects were mimicked by epidermal growth factor (EGF), insulin (In), insulin-like growth factor I (IGF-I) and basic fibroblast, alone and/or in combination. These results provide a morphological and functional basis supporting LHRH-astroglia interactions and further underscore the importance of growth factors not only in regulating axon growth on astroglia in vitro but also contributing to signal transduction involved in the regulation of LHRH release.

742.2

KAINATE GLUTAMATE RECEPTORS IN TANYCYTES AND TYPE II ASTROCYTES OF THE RAT ARCUATE NUCLEUS. F. Naftolin[1], S. Diano[1], J. Lerma[2]*, L.M. Garcia- Segura[2], C. Leranth[1] and T.L. Horvath[1]. [1]Yale University, Dept. of Ob/Gyn, New Haven CT, 06520, and [2]Instituto Cajal, Madrid, 28002, Spain.

It has been suggested that neuro-glial interactions play important roles in the central regulation of neuroendocrine processes. For example, estrogen-sensitive tanycytes that ring the third ventricle and extend to the median eminence providing morphological organization of the area, have been implicated in the regulation of neurotransmitter and peptide release into the pituitary portal vessel. Furthermore, type II astrocytes were demonstrated to participate in the estrogen-induced synaptic remodelling of arcuate neurons. Since glutamate is a dominant neurotransmitter in the neuroendocrine hypothalamus, we tested the possibility that tanycytes and type II astrocytes in the arcuate nucleus express receptors for glutamate offering a possible mechanism for neuro-glial crosstalk. **Experimental:** Single label immunocytochemistry for different ionotropic glutamate receptors (GluR 1-7, NMDAR 1-2) were carried out on sections containing the rat arcuate nucleus. The avidin-biotin-peroxidase method was used to visualize the immunolabeled profiles. While all of the antibodies were found to label neurons in the hypothalamus, only labeling for GluR 5-7 resulted in immunopositivity in glial elements. From the retrochiasmatic area to the mammillary bodies, heavy peroxidase staining of tanycytes and astrocytes could be observed in the arcuate and periventricular nuclei and th emedian eminence. These findings were confirmed by electron microscopy. **Conclusions:** The observation of immunoreactive kainate glutamate receptors in specific hypothalamic glial elements indicates a hitherto unexplored division of glutamate function in the hypothalamus. This may provide an underlying mechanism for neuro-glial interactions which are implicated in hypothalamic synaptic plasticity and regulation of neuroendocrine functions. (Support: NIH Grant HD13587 to F.N., Brown-Coxe Fellowship to T.L.H.)

742.3

PERSISTENT INDUCTION OF GALANIN GENE EXPRESSION IN GnRH NEURONS FOLLOWING THE LH SURGE IN RATS. P.D. Finn,* M.R. King, D.K. Clifton, R.A. Steiner. Ob/Gyn & PBio, University of Washington, Seattle, WA 98195.

In female rats, galanin is a cotransmitter in GnRH neurons, and its expression is regulated by sex steroids. During the preovulatory LH surge, levels of galanin mRNA in GnRH neurons are elevated; however, the full time-course of the rise and decline of galanin mRNA in GnRH neurons with respect to the LH surge is unknown. To address this issue, we measured and compared levels of galanin mRNA in GnRH neurons and plasma levels of LH during a 30 h period bracketing the LH surge, beginning 6 h before and continuing for 24 h after a steroid-induced surge. Ovariectomized rats were treated with estradiol benzoate on Day 0 at 1030 h and progesterone on Day 2 at 1200 h to induce an LH surge (lights on at 0700 h and off at 2100 h), and groups (n=4-6/time point) were sacrificed at 1200, 1400, 1500, 1600, 1800, and 2400 h (Day 2) and 0600, 1200, and 1800 h (Day 3). GnRH neurons were identified with a digoxigenin-labeled GnRH cRNA probe, and galanin mRNA was simultaneously measured in the GnRH neurons with an ^{35}S-UTP-labeled cRNA probe and a computerized image analysis system. Results showed that the LH surge began at 1600 h on Day 2, peaked at 1800 h, and returned to baseline by 2400 h that day. On Day 2, levels of galanin mRNA levels in GnRH neurons were low between 1200 and 1600 h (18 ± 2 to 27 ± 2 grains/cell), increased by two-fold by 1800 h (46 ± 10 grains/cell; $p < 0.05$ vs. 1600 h), by 4-fold by 2400 h (80 ± 6 grains/cell; $p < 0.05$ vs. 1600 h), and remained elevated at all time points on Day 3 (75 ± 5 to 80 ± 7 grains/cell). **Conclusion:** The induction of galanin mRNA in GnRH neurons persists for at least 24 h following the transient LH surge, indicating that galanin may serve a physiological role in events that *follow* the surge itself, e.g. behavioral estrus.

742.4

GALANIN AND NEUROPEPTIDE Y IMMUNOREACTIVITY IN HYPOTHALAMIC NUCLEI IN RELATION TO THE ESTROUS CYCLE. J.T. Alexander*, A. Akabayashi, S.M. Gabriel, L.E. Baskin, C.J. Owen and S.F. Leibowitz. The Rockefeller University, New York, N.Y., 10021.

The hypothalamic peptides, galanin (GAL) and neuropeptide Y (NPY), are known to change in relation to circulating gonadal steroids, in an anatomically- and gender-specific manner, and to control the secretion of pituitary hormones. This relationship, between gonadal steroids and brain peptides, was further investigated in relation to the estrous cycle. Female rats (n=32) were sacrificed at dark onset at different stages of the estrous cycle, and trunk blood was collected. Peptide levels were measured (via RIA) in 10 hypothalamic areas: medial (mPVN) and lateral (lPVN) portions of the paraventricular nucleus, median eminence (ME), medial preoptic nucleus (MPO), the arcuate (ARC), dorsomedial, suprachiasmatic, and supraoptic nuclei, ventromedial and lateral hypothalamic areas. During proestrous, when luteinizing hormone surges ($p<0.01$), GAL levels are found to peak sharply in a site-specific manner. This peak occurs in the MPO (+36%, $p<0.05$), lPVN (+62%, $p<0.05$) and ME (+42%, $p<0.05$), and is followed by a decline to low levels at metestrous. These shifts in endogenous GAL may be distinguished from those of NPY. This peptide rises during proestrous, specifically in the ARC (+73%, $p<0.05$), mPVN (+36%, $p<0.05$), and MPO (+58%, $p<0.05$); during metestrous, NPY declines to low levels in the ARC and mPVN. Other hypothalamic areas exhibit no temporal shifts in the peptides. These results provide indirect support for strong activational effects of gonadal steroids, during proestrous, on GAL and NPY production in specific brain sites.

742.5

PHENOTYPIC CHARACTERIZATION OF A POPULATION OF PREOPTIC AREA NEURONS ACTIVATED DURING AN LH SURGE W.-W. Le, K.A. Berghorn, H.-J. Wang and G.E. Hoffman*. Department of Neurobiology, Univ. Pittsburgh, Pittsburgh, PA, 15261.

The periventricular zone of the preoptic area (pePOA) is an area which sends a projection to the vicinity of the LHRH neurons. At the time of an LH surge, pePOA neurons are activated in synchrony with LHRH neurons. Previous studies from our laboratory identified a dopaminergic subcomponent of pePOA neurons, but the phenotypes of the remaining neurons in the area were still unknown. In the present study, we explored further the phenotypic characterization of the pePOA neurons using immunocytochemical and *in situ* hybridization procedures. With immunocytochemistry, we observed that while only a small fraction (less than one third) of the pePOA neurons expressed tyrosine hydroxylase, more than half of the pePOA neurons activated on proestrus contained the l-amino acid decarboxylase. Additionally, *in situ* hybridization (ISH) for galanin mRNA with biotinylated riboprobes using immunocytochemistry (ICC) for biotin visualization in free-floating acrolein-paraformaldehyde fixed brain sections revealed that galanin was found not only within the LHRH neurons at the time of an LH surge, but also in many neurons of the pePOA. Double labeling of galanin mRNA combined with ICC staining of cFos showed that galanin mRNA was detected in cFos-positive pePOA neurons at the time of an LH surge. These data suggest that multiple effectors are present in the activated pePOA neurons and that while galanin within LHRH neurons may provide one means of affecting LHRH function, activation of afferent galanin neuron populations may contribute to the LH surge as well. (*Supported by NIH NS 28730*)

742.6

PHARMACOLOGICAL ANALYSIS OF NEUROPEPTIDE Y (NPY) RECEPTORS MEDIATING NPY FACILITATION OF LH SURGES. S.M. Leupen, C. Berg, and J.E. Levine*, Dept.of Neurobio. & Physiology, Northwestern Univ., Evanston, IL 60208

We previously showed that NPY facilitates LHRH-induced LH surges, and that endogenous NPY is critical for normal elaboration of preovulatory gonadotropin surges. The NPY receptor subtype mediating this action has not been identified. Three subtypes have been described, based on rank order of affinities for NPY receptor agonists: Y1 (PYY≥NPY≥Y1-a>>Y2-a), Y2 (PYY> NPY >Y2-a>>Y1-a), and Y3 (NPY≥ Y1-a≥Y2-a>>PYY) receptors. These experiments were designed to pharmacologically characterize anterior pituitary receptors which mediate NPY's actions on LH secretion. Adult female rats were prepared for peptide infusion and blood sampling on proestrus. Animals received pentobarbital at 1330h to block endogenous LHRH release, and thereafter received pulsatile infusions of LHRH paired with saline or one of four NPY agonists: NPY, PYY, [$Leu^{31}Pro^{34}$]NPY (Y1-a), NPY(13-36)(Y2-a). Each rat received pulse infusions containing either LHRH (15ng/pulse) or LHRH (15ng/pulse) & agonist (10 ug/pulse) at 30min intervals from 1400-1800h. Hourly blood samples were stored for LH RIA. As previously shown, NPY significantly augmented the LH response to LHRH ($p<.03$). Both PYY and Y1-a also significantly potentiated LHRH-induced LH surges, while the Y2-a was without effect. The rank order of potency was found to be PYY = Y1-a > NPY >>> Y2. These findings implicate a Y1 or Y1-like receptor subtype in the mediation of NPY's facilitatory effects on LH secretion. In current work we are attempting to determine if this pituitary NPY receptor is identical with the cloned Y1 receptor expressed in other tissues, or if it is a variant with Y1-like pharmacology.

742.7

EFFECTS OF ESTRADIOL (E2) AND PROGESTERONE (P4) ON PROOPIOMELANOCORTIN (POMC) AND NEUROPEPTIDE Y (NPY) GENE EXPRESSION IN THE ARCUATE NUCLEUS: A POSSIBLE ROLE IN THE ABILITY OF PROGESTERONE TO SEQUENTIALLY ENHANCE AND THEN INHIBIT THE LH SURGE. P.M. Wise*[1], A. Cai[2], K. Scarbrough[3]. [1]Department of Physiology, University of Kentucky, Lexington, KY 40536 and [2]Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201 and [3]Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

POMC and NPY neurons, whose cell bodies are located in the arcuate nucleus, are thought to play roles in the cyclic release of LH. We tested the hypothesis that changes in the gene expression of these neuropeptides can explain the ability of P4 to enhance the LH surge induced by E2 on the day that it is administered and then to inhibit the surge on the following day. Rats were ovariectomized and 1 week later were implanted with Silastic capsules containing E2. Two days later (day 2), animals received an i.p. injection of P4 (1.5 mg/rat) or sesame oil at 1000h. Using this protocol, E2 induces an LH surge during the afternoon of day 2 and 3; P4 enhances the surge on day 2 and suppresses the surge on day 3. E2-treated rats were killed at 0600, 1400, 2300 on day 2, 0600 and 1400h on day 3. E2P4-treated rats were killed at 1400, 2300 on day 2 and 0600 and 1400h on day 3. The anterior and posterior arcuate nuclei were dissected, RNA was extracted and POMC and NPY mRNA levels were quantitated by solution hybridization/ RNase protection using ^{32}P-labelled cRNA probes. E2 induced a rhythm in POMC gene expression in the anterior arcuate nucleus: levels reached their nadir at 2300h; P4 prevented this decrease. NPY mRNA levels in the anterior arcuate nucleus appeared to be lowest at 0600h and to increase during the day. P4 increased NPY mRNA levels at 0600h on day 3. Thus, POMC and NPY may play a role in the suppression of LH surges the day after P4 is administered (Supported by NIH AG02224 to PMW).

742.8

CENTRAL VIP-ANTISERUM INJECTIONS ALTER THE TIMING OF AN ESTROGEN-INDUCED LH SURGE E.M. van der Beek*, J.J.M. Swarts and V.M. Wiegant Dept Human & Anim Physiol, Agricult Univ, Wageningen & Rudolf Magnus Inst Neurosci, Utrecht University, Utrecht, The Netherlands

Lesion and tracing studies of the suprachiasmatic nucleus (SCN) have suggested the exisistence of a vasoactive intestinal polypeptide (VIP)-containing pathway between the SCN and the gonadotropin-releasing hormone system (GnRH). Recently, we provided evidence for the involvement of this pathway in the activation of the GnRH system during the LH surge (Van der Beek et al, Endocrinology 1994 134;2636).

In the present study, we investigated the effect of intracerebroventricular VIP-antiserum (VIP-Ab) injections on the timing and height of an estrogen (E)-induced LH surge in mature ovariectomized (OVX) female rats. Wistar rats (N = 20) were housed under LD12:12 (lights on from 2:00-14:00) and OVX on day 0. The LH surge was induced by two consecutive sc E-injections on day 7 and 8. Animals received two saline or VIP-Ab injections (day 8; 22:00 h, day 9; 8:00 h), and hourly blood samples were collected from 9:00 to 18:00 on day 9 and assayed for LH. Two weeks later, rats were used in a second experiment and treatments were reversed (VIP-Ab or saline). During the second experiment, rats were sacrificed between 15:00-16:00 h, and the activation of the GnRH system was evaluated using c-fos immunocyto-chemistry. VIP-Ab treatment delayed the onset as well as the peak of the LH surge for 1 to 2 h, and significantly reduced the height of the surge. The percentage of c-fos-activated GnRH cells in saline animals (39.9 ± 6.5%) did not differ from that in VIP-Ab treated females (43.8 ± 8.3%), and was comparable with that previously found in E + P-treated animals.

These data demonstrate that blockade of central VIP-activity alters the timing as well as the height of the E-induced LH surge, but does not affect the activation of the hypothalamic GnRH system.

742.9

IMMUNOHISTOCHEMICAL LOCALIZATION OF GONADOTROPIN RELEASING HORMONE, GLUTAMATE AND KAINATE-2 RECEPTOR PROTEIN IN RAT BRAIN. O. Eyigor* and L. Jennes. Dept. Anatomy & Neurobiology, Univ. Kentucky, Coll. Medicine, Lexington, KY 40536

Glutamate is the most abundant neurotransmitter in the brain and has been implicated to participate in the regulation of gonadotropin releasing hormone (GnRH) release. The first aim of the present study was to determine if glutamate is contained in axon terminals which could innervate GnRH neurons at their perikarya or at their nerve terminals in the median eminence. Immunofluorescence double stainings for GnRH and glutamate show that glutamate is present in numerous punctae juxtaposed to GnRH perikarya as well as to GnRH containing axons in the median eminence suggesting that, from an anatomical point of view, glutaminergic neurons can provide axo-somatic and axo-axonal input to GnRH neurons. The effects of glutamate are mediated through activation of 2 families of receptors, the ionotropic and the metabotropic receptors. The second aim of the present study was to determine if GnRH neurons express the kainate receptor and where in the GnRH neurons the receptor protein is located. Immunofluorescence double stainings for GnRH and kainate-2 receptors show that some but not all GnRH perikarya in the medial septum-diagonal band and GnRH-containing axon terminals in the median eminence contain immunoreactive kainate-2 receptor protein. Together, the results suggest that glutaminergic axons are in an appropriate position to provide excitatory input to the GnRH neuronal system and that some, but probably not all of this input is mediated by activation of kainate-2 receptor subtypes. Supported by NIH HD 24697 (LJ).

742.10

PULSATILE NOREPINEPHRINE (NE) AND LH RELEASE IN OVARIECTOMIZED (OVX) RATS. S. J. Legan.* Department of Physiology, University of Kentucky, Lexington, KY 40536.

In OVX monkeys, NE and LHRH pulses in the stalk-median eminence are coincident (Terasawa et al., 1988), suggesting that NE neurons projecting to the median eminence control pulsatile LH secretion and constitute part of the LHRH pulse generator. In rats, however, the majority of LHRH cells are located in the septum/ diagonal band area (S/DB) of the hypothalamus. Therefore, to examine the correlation between NE release in the S/DB and LH pulses in OVX rats, NE release was determined in microdialysates of the S/DB which were obtained every 5 min (2 μl/min) for 2 hours beginning at 1000 h four weeks after ovariectomy. Blood samples (0.1 ml) were also collected every 5 min from a right atrial cannula. Placement of microdialysis probes was verified histologically. Pulses of LH and NE were detected using the ULTRA software. Plasma LH concentrations, determined by radioimmunoassay, averaged 17.62 ± 2.73 ng/ml (X ± SE, n=6) and were characterized by a frequency of 1.92 ± 0.30 pulses per hour. NE release, determined by HPLC, averaged 34.09 ± 8.18 pg per fraction (n=6) and NE pulse frequency was 2.58 ± 0.37 per hour. Only 22% of the NE pulses, however, occurred within 5 min before, or coincident with, LH pulses. These results indicate that in OVX rats, each NE pulse does not stimulate a corresponding LH pulse, thus the noradrenergic neurons that project to LHRH cells in the S/DB may not be an intrinsic part of the LH pulse generator in the rat.

742.11

IMMORTALIZED LHRH CELLS (GT1-7) EXPRESS TYPE I PITUITARY ADENYLYL CYCLASE-ACTIVATING PEPTIDE (PACAP) RECEPTORS. J. Olcese*[a], R. Middendorff[b] and C.A. McArdle[c]. Inst. for Hormone & Fertility Research[a], Inst. of Anatomy.[b], Univ. of Hamburg, 22529 Hamburg, Germany; Dept. of Medicine[c], Univ. of Bristol, Bristol BS2 8HW, U.K.

The regulation of LHRH release involves signal transduction pathways often linked to the elevation of cyclic nucleotide production. A potent stimulator of cyclic AMP accumulation is the peptide PACAP, whose specific receptor has recently been cloned. The goals of the present investigation were to determine whether PACAP can activate cAMP production and/or LHRH release from GT1-7 cells, and whether these cells express the type I PACAP receptor. Both PACAP-38 and its truncated form PACAP-27 were potent in elevating cAMP. Similar experiments revealed that these peptides elevated LHRH release 2-3 fold within 10 minutes of treatment, with an EC50 in the low nM range. Six splice variants of the type I PACAP receptor have been described - the short form and five additional forms having 1 to 2 inserts in the third intracellular loop. RT-PCR using primers encompassing this region revealed the presence of the short transcript and a single insert transcript in GT1-7 cells. On the basis of these results, we propose that PACAP may be important not only via its actions at the pituitary gonadotrope level but also via the direct regulation of LHRH secretion.

742.12

DO LHRH NEURONS OF MALE SYRIAN HAMSTER EXPRESS GLUTAMATE RECEPTORS? H.F. Urbanski, V.T. Garyfallou, S.G. Kohama, G. Munro* and C. Lee. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Although excitatory amino acids (EAAs) can profoundly stimulate the hypothalamo-pituitary-testicular axis of male Syrian hamsters it is unclear whether they directly influence the secretory activity of luteinizing hormone-releasing hormone (LHRH) neurons. In the present study adult male hamsters *(Mesocricetus auratus)* were either maintained in a reproductively active phase under long days or were induced to revert to a sexually quiescent condition by 12 weeks of exposure to short days. Two different double-labeling histochemical techniques were then performed on 4%-paraformaldehyde-fixed coronal brain sections (30 μm) in order to disclose whether the LHRH neurons of hamsters express glutamate receptors. The first technique involved double-label fluorescence microscopy; the sections were labeled sequentially with an FITC-tagged monoclonal LHRH antibody and then with rhodamine-tagged polyclonal antibodies to either GluR1 or NMDAR1 (i.e. two glutamate receptor subtypes). In the second technique, the sections were sequentially processed for LHRH immunocytochemisty and then *in situ* hybridization histochemistry using RNA probes to either GluR1 or NMDAR1. In total, 829 LHRH neurons were examined for colocalization of either GluR1 protein or mRNA but in no instance was double-labeling of the LHRH neurons observed, regardless of the animals photoperiodic history or reproductive status. Similarly, in preliminary observations there did not appear to be any obvious expression of NMDAR1 protein nor mRNA in the LHRH neurons. Taken together, these results do not support the hypothesis that EAA's primary influence on the reproductive axis of adult hamsters is mediated by a direct stimulatory action on the LHRH neurons but, rather, suggests that interneurons may be involved. *Grant support: HD-24312, HD-29186, RR-00163 and NSF 93-09368.*

742.13

DISTRIBUTION OF FOLLISTATIN mRNA IN THE RAT BRAIN: IMPLICATIONS FOR A ROLE IN THE REGULATION OF CENTRAL REPRODUCTIVE FUNCTIONS. L.A. MacConell*, S. Barth and V. J. Roberts. Dept. Repro. Med., UCSD, La Jolla, CA 92093

Follistatin (FS) is a glycosylated single-chain protein originally isolated from porcine follicular fluid. FS binds to the inhibin/activin βA or βB subunit, therefore allowing activin two binding sites for FS and inhibin only one (Shimonaka et al. 1991; Endocrinol. 128:3313). This association is consistent with the apparent specific action of FS on the regulation of activin-mediated effects. The tissue specific localization of FS gene expression generally coincides with that of activin subunit expression (Roberts and Barth 1994; Endocrinol. 134:914). However, in contrast to the wide distribution of activin subunit proteins and mRNAs in the brain, weak FS mRNA signal has only been observed in the olfactory tubercle and layer II of the frontal cortex (Shimasaki et al. 1989; Mol. Endocrinol. 3:651). We hypothesized that central FS gene expression is more widely distributed and localized in regions coinciding with inhibin/activin β subunits and possible activin-mediated effects.

The present investigation utilized *in situ* hybridization with a ^{33}P-labeled RNA probe specific for rat follistatin. Abundant FS mRNA is expressed in mitral cells of the olfactory bulb and layer II and IV of the frontal cortex. In addition, FS mRNA signal was observed in hypothalamic regions where GnRH neurons are localized (nucleus of the diagonal band), in areas associated with the activin-oxytocin pathway (solitary tract nucleus, paraventricular nucleus), and in aspects of the brain with abundant βA immunostaining in cell nuclei (striatum, medial vestibular nucleus). These results suggest that FS is localized in sites compatible with a role in the regulation of central reproductive functions.

742.14

AGE RELATED CHANGES IN THE ABILITY OF ESTROGEN TO INDUCE PROGESTERONE RECEPTOR (PR) mRNA IN FEMALE RATS. G.M. Hejna, A.H.Nagahara*, R.F. Mcgivern+, Y. Li and R.J. Handa. Dept of Cell Biology Neurobiology and Anatomy Loyola University,. Chicago, Stritch School of Medicine. Maywood, IL 60153 and +Dept. of Psychology, San Diego State University, San Diego, CA 92120.

Previous studies have shown that PR mRNA and PR binding increases following administration of estrogen (E). These increases correlate with the ability of progesterone to induce female reproductive behaviors. In this study, we examined age related changes in the ability of estrogen to induce PR mRNA in the medial preoptic area (MPOA), ventromedial hypothalamus (VMH) and arcuate nucleus (ARC). PR mRNA was measured by *in situ* hybridization with a ^{35}S labelled cRNA probe (rat PR cDNA template kindly provided by Dr. J. Kato) Female rats of 2-3, 7-8 and 15-17 months of age were ovariectomized and one week later injected with 17β estradiol (0.5ug in sesame oil) or sesame oil in the A.M. for three consecutive days prior to sacrifice. Animals were sacrificed in the evening following the third injection. Estrogen treatment significantly ($p<0.01$) increased PR mRNA when compared to controls in all three brain regions and at all ages. In the MPOA of 15 month old rats there was a significant ($p<0.05$) decrease in the ability of E to induce PR mRNA. In the VMH of 15 month old rats there was a significant increase in the ability of E to induce PR mRNA ($p<0.01$) There was no age related effects in the ARC. However, cohorts of these animals showed no age related deficits in lordosis quotient in response to estrogen and progesterone treatment. These data demonstrate that there are region specific effects of age on the ability of E to induce PR mRNA. The importance of these changes in the ability of progesterone to reduce female reproductive behavior remains to be determined. Supported by NSF IBN 94-08890 (RJH) USPHS AA008696 (RJH) and AA06478 (RFM).

742.15

DEVELOPMENT OF AN OVINE Y3 cDNA AND EXPRESSION OF THE Y3 RECEPTOR mRNA IN THE OVINE HYPOTHALAMUS AND PITUITARY. C.J. Dyer[1], R.M. Matteri[2] and D.H. Keisler[1]. [1]Department of Animal Science, University of Missouri, Columbia; [2]Animal Physiology Research Unit, Agricultural Research Unit, USDA, Columbia, Missouri 65211.

Neuropeptide Y (NPY) has been shown to exert effects upon the hypothalamic-pituitary-gonadal axis, but the mechanisms through which this peptide acts remain unclear. We report here the development of an ovine cDNA to the putative NPY receptor Y3 (also called LCR1). Ovine hypothalamic RNA was used in RT-PCR with primers derived from the published bovine Y3 sequence to amplify a 578 base pair cDNA. Cloning and sequencing revealed a >90% homology to the corresponding bovine sequence. Radiolabeled riboprobes derived from this ovine cDNA were used in a ribonuclease protection assay (RPA) to detect Y3 mRNA in ovine anterior and posterior hypothalamic and pituitary RNA extracts. High levels of Y3 mRNA expression were consistently detected in the pituitary extracts; much lower levels were detected in the posterior hypothalamus (containing the arcuate nucleus). No expression was detected by RPA in the anterior hypothalamus (containing the pre-optic area). The RNA extracts were then subjected to RT-PCR and Southern hybridization. Expression of Y3 mRNA was detected in all samples, demonstrating that low levels of Y3 mRNA, undetectable by RPA, exist within the anterior hypothalamus. These data suggest that the Y3 receptor mRNA is only weakly expressed in the hypothalamus but strongly expressed in the pituitary. Work is now underway to determine if gonadotrophs within the pituitary express Y3 mRNA.

742.16

ESTRADIOL REGULATION OF NITRIC OXIDE SYNTHASE mRNAs IN RAT HYPOTHALAMUS. S. Ceccatelli *[1,2], R. Scott[1], L. Grandison[1], D.W. Pfaff[1], L.-M. Kow[1]. [1]Lab. of Neurobiology and Behavior, The Rockefeller University, New York, NY 10021 and [2]Inst. of Environmental Medicine, Karolinska Institute, S-171 77 Stockholm, Sweden.

We have used *in situ* hybridization to investigate estradiol regulation of the mRNAs of neuronal, endothelial and macrophage forms of the nitric oxide (NO)-synthesising enzymes NOS. Our study focused on regions of the hypothalamus which contain estrogen receptors and are related to specific neuroendocrine functions. Ovariectomized (OVX) rats were treated with vehicle or 3 μg/100 g estradiol benzoate (EB) for 7 days. Brains were sectioned and hybridized with ^{35}S-antisense riboprobes for the mRNAs of the 3 NOS isoforms. Sections were exposed to X-ray film and/or autoradiographic emulsion. Only the neuronal NOS mRNA was clearly detectable in the hypothalamic regions examined with a strong hybridization signal in supraoptic, paraventricular (PVN) and ventromedial (VMN) nuclei. Quantitative analysis showed a 3 fold increase in neuronal NOS mRNA in the VMN of the OVX rats treated with EB. The increase was restricted to the ventrolateral aspect of the VMN. No significant changes were observed in the hypothalamic PVN. These data suggest that the expression of neuronal NOS mRNA in VMN is regulated by estrogen. Moreover, since VMN is an integral part of the neural circuitry controlling lordosis, NO may play a role in female sexual behaviors.

742.17

HORMONAL REGULATION OF PHOSPHORYLATION OF CREB IN THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS (AVPV). G. Gu*, A. A. Rojo , M.C. Zee, J. Yu and R.B. Simerly. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

The AVPV is a nodal point in neural circuits regulating secretion of gonadotropin and contains sexually dimorphic populations of dopaminergic and dynorphin containing neurons. Despite clearly documented effects of estrogen on TH and PDYN gene expression in the AVPV, consensus estrogen response elements have not been identified in these genes, suggesting that the observed regulatory patterns are not due to a direct action of the hormone-bound receptors on transcriptional activation of TH or PDYN. However, both the TH and PDYN genes contain sequences in their promoters that bind transacting factors thought to mediate the actions of calcium and cAMP, such as the cAMP response element binding protein (CREB). The present histochemical study was undertaken to determine if estrogen alters expression of CREB in the AVPV, and since the ability of CREB to activate transcription is dependent on phosphorylation of Ser^{133}, we also evaluated the effects of acute estrogen treatment on levels of phosphorylated-CREB (PCREB) in AVPV neurons by using an antibody that differentiates between CREB and PCREB. Ovariectomized rats were perfused at 5, 15, 30 mins, and 1, or 4 hrs. after treatment with exogenous estradiol. Although these treatments did not alter CREB immunostaining in the AVPV, a significant induction in the number of PCREB-immunoreactive nuclei was observed within 30 min. of treatment and was maintained for at least 4 hrs. Pretreatment with the estrogen antagonist nafoxidine blocked this induction. In contrast, acute administration of progesterone to estrogen primed animals suppressed and then increased PCREB staining in the AVPV at 30 and 60 minutes, respectively; no significant differences between experimental and control animals were apparent by 2 hrs. after progesterone treatment. Double labeling experiments are being used to determine if PCREB is preferentially regulated in TH or PDYN neurons.

742.18

HORMONAL REGULATION OF SUBSTANCE P RECEPTOR (NK-1) mRNA IN THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS OF THE RAT. M.C. Zee, G.B. Gu, J.E. Krause, and R.B. Simerly*. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006 and Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The anteroventral periventricular nucleus (AVPV) of the preoptic region is a sexually dimorphic cell group that plays a critical role in the control of gonadotropin secretion. It contains high densities of receptors for sex steroid hormones and receives strong sexually dimorphic substance P (SP) containing inputs from the principal bed nucleus of the bed nuclei of the stria terminalis (BSTp) and posterodorsal part of the medial nucleus of the amygdala (MeApd). Although both SP, the BSTp and MeApd appear to play stimulatory roles in the neural control of gonadotropin secretion, cellular levels of SP are not acutely upregulated in the BSTp or MeApd. The present in situ hybridization study was undertaken to examine the possible hormonal regulation of neurokinin-1 (or SP) receptor (NK-1R) gene expression in the AVPV. Levels of NK-1R mRNA fluctuate during the estrous cycle from a minimum during metestrus to maximum levels during proestrus. The correspondence between this regulatory pattern and that of circulating estrogen is supported further by the observation that treatment of ovariectomized rats with estradiol results in a 2 fold increase in NK-1R mRNA in the AVPV. Short term (3 hrs.) treatment of estrogen primed ovariectomized rats with progesterone did not alter levels of NK-1R mRNA in the AVPV, but after an additional 24 hrs NK-1R levels fell by approximately 40%. These findings suggest that the impact of SP on AVPV neurons is greatest on the morning of proestrus, when estradiol levels are greatest. Elevated levels of progesterone that characterize the afternoon of proestrus, together with reduced levels of estradiol, lead to diminished expression of NK-1R s on the day of estrus, and presumably reduced responsiveness of AVPV cells to the release of SP from neurons in the BSTp and MeApd.

742.19

EXPRESSION OF NUCLEAR TRANS-ACTING FACTORS IS REGULATED DIFFERENTIALLY BY OVARIAN STEROIDS IN THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS (AVPV) OF THE RAT. J. W. Pendleton*, A. M. Carr, M.C. Zee, and R B. Simerly. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

The AVPV represents an attractive model system for identifying cellular factors involved in neuroendocrine regulation of hypothalamic neurons because it plays a critical role in the neural control of gonadotropin secretion and contains sexually dimorphic populations of neurons that display distinct patterns of transmitter gene expression. In situ hybridization was used to examine the expression of mRNAs encoding nuclear trans-acting factors in the AVPV, including the estrogen (ER) and progesterone (PR) receptors as well as the protooncogenes cfos, cjun and jun B. Estradiol treatments suppressed expression of ER mRNA in the AVPV, and increased levels of PR mRNA 5-fold. Acute treatment of estrogen-primed ovariectomized rats with progesterone (3 hrs.) increased levels of ER mRNA in AVPV neurons, but did not affect PR mRNA expression, although 27 hrs. after progesterone treatment PR mRNA appears to decline in the AVPV. ER gene expression fluctuated during the estrous cycle with minimal levels observed on the afternoon of proestrus, and the highest levels during metestrus. In contrast, PR mRNA levels were highest during diestrus and lowest on the morning of proestrus suggesting that PR expression in the AVPV is regulated in a complex manner that does not correspond to circulating ovarian steroid hormones. The expression of cfos mRNA in the AVPV also fluctuates over the estrous cycle with maximal levels occurring on the morning of proestrus and with significantly lower levels on the day of metestrus. Preliminary work indicates that acute administration of estradiol may induce c-fos mRNA in the AVPV. In addition, the protooncogene cjun is expressed in the AVPV, but displays no apparent regulation during the estrous cycle, and jun B is undetectable in the AVPV at any point during the cycle.

HYPOTHALAMIC-PITUITARY-GONDAL REGULATION V

743.1

RU486 ADMINISTRATION ATTENUATES LH SURGES IN THE ABSENCE OF ENDOGENOUS PROGESTERONE. P.E. Chappell, T. Porkka-Heiskanen and J.E. Levine. Dept. of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

Progesterone (P) amplifies and advances LH surges in estrogen-primed rats, and pharmacological blockade of P receptors (PRs) greatly attenuates spontaneous preovulatory LH surges in proestrous rats. Measurements of P during the afternoon of proestrus, however, have revealed that little secretion of the steroid occurs prior to the onset of the LH surges. It has therefore been proposed that ligand-independent activation of PRs, which has been demonstrated in vitro (Turgeon & Waring, 1994) may instead function as a component of the surge-generating process. To test this hypothesis in vivo, adult female rats were ovariectomized (OVX) on diestrus 2 and administered an s.c. injection of estradiol benzoate (EB; 30ug) sufficient to stimulate LH surges on the following day of presumptive proestrus. Additional animals were adrenalectomized (ADX) at the time of OVX, ruling out actions of adrenal P. On presumptive proestrus, rats received either RU486 (6mg/kg s.c.) or oil at 1230h and were killed at 1730h. RIA of LH in trunk blood revealed that EB stimulated LH surges in OVX and OVX & ADX rats which subsequently received oil. Administration of RU486, however, significantly attenuated LH levels in both groups by 50%. Since endogenous P was eliminated in these animals, our data suggest that RU486 blocks ligand-independent activation of PRs which may normally occur as an important component of the LH surge-generating process. The role of ligand-independent activation of PRs in the generation of LH surges remains to be confirmed by further pharmacological analysis. In current work we are also attempting to characterize the anatomical distribution of hypothalamic and/or pituitary PRs which may be activated towards this end. (Supported by NIH R01-HD20677, P30-HD28048, P01-HD21921).

743.2

ESTRADIOL INCREASES FOS EXPRESSION IN A SUBSET OF ESTROGEN RECEPTOR-CONTAINING NEURONS IN THE PREOPTIC AREA. B.A. Adrian*, H.T. Jansen#, M.N. Lehman# and R.L Goodman. Dept. Physiology, West Virginia Univ., Morgantown, WV 26506 and #Dept. Cell Biology, Neurobiology and Anatomy, Univ. Cincinnati, Cincinnati, OH 45267.

In the ewe, estradiol (E) inhibits luteinizing hormone (LH) pulse frequency during anestrus but not the breeding season. Previous studies have demonstrated a role for dopaminergic cell groups in this effect of E. However, since neither these dopamine cell groups, nor gonadotropin-releasing hormone neurons possess estrogen receptors (ER), another neural system must mediate E action. As a first step to identify this system, we examined the effect of E treatment on the expression of the immediate early gene product, Fos (a marker of neuronal activation), in cells that also contain ER. Ovariectomized ewes were implanted with either a blank (n=3), or E filled (n=3), 0.5 cm Silastic capsule during anestrus to suppress pulse frequency without inducing an LH surge. Seven days later animals were bled for 6 hours at 12 min. intervals and then sacrificed. After fixation, brains were removed, sectioned and stained for ER and Fos using a dual immunofluorescence procedure. E treatment inhibited LH pulses and significantly increased the percentage of ER-containing neurons expressing Fos (2.07% vs 16.75%) in the rostral preoptic area (POA), but not in other regions of the POA. E had no effect on colocalization of Fos and ER in any other hypothalamic area (AHA, MBH, ARC). E also did not alter the number of neurons containing ER in any area examined. These data suggest that a subset of ER-containing neurons in the POA are involved in the inhibition of LH pulse frequency by E during anestrus. (Supported by NIH HD17864 and HD21968)

743.3

SEX DIFFERENCES IN SERUM LUTEINIZING HORMONE (LH) RESPONSE TO GONADECTOMY ARE NOT EXPLAINED BY CHANGES IN mRNA FOR GONADOTROPIN RELEASING HORMONE (GnRH) OR THE SECRETAGOGUE INDUCED RELEASE OF GnRH A.A. Elskus, S.C.H. Hood, and N.B. Schwartz*. Dept. of Neurobiology and Physiology, Northwestern University, Evanston IL 60208

A continuing puzzle in reproductive endocrinology is the striking sex difference in serum LH response to gonadectomy (gnx); male levels rise within hours, while levels in females do not rise significantly for several days. We have ruled out sex differences at the pituitary level. We measured GnRH transcription and release as a possible source for this sex difference. *In situ* hybridization was performed using brains from intact, 12, and 24 h post-gnx male and female rats. Serum LH in the males was significantly elevated by 12 h; levels in females had not changed by 24 h. The average number of GnRH mRNA expressing cells, as well as the intensity, rose by 12 h post-gnx in both males and females and remained elevated or began to decline by 24 h. These data demonstrate that the difference in initial rate of serum LH rise in gnx males (rapid) versus females (slow) is not reflected in differences in the early timing of GnRH mRNA synthesis. GnRH release was measured *in vitro* from perifused median eminences of intact and 24 h post-gnx male and female rats. Tissue was stimulated with NMA, and the resulting GnRH release was measured by RIA. No significant differences were found in GnRH release between groups. This does not rule out sex differences in hypothalamic innervation or other regulatory factors such as NPY, not present when the tissue is isolated *in vitro*. Together these data suggest that an upstream sex difference, yielding differences in pulse frequency or amplitude of GnRH release, is likely responsible for sex differences in LH release following gnx. Supported by NRSA 1-F32-HD07657 (to AAE), PO1 HD-21921 and P30 HD28048.

743.4

CHANGES IN DOPAMINE CONCENTRATION AND ACTIVITY OF HYPOTHALAMIC DOPAMINERGIC NEURONS DURING PROLACTIN SURGES IN PSEUDOPREGNANT RATS A. Lerant, M.E. Herman & M.E. Freeman* *Department of Biological Science , Florida State University, Tallahassee, FL 32306*

The role of tuberoinfundibular dopaminergic (TIDA) neurons originating from the caudal arcuate nucleus (ARN) in modulating prolactin (PRL) secretion is well established. However, the significance of tuberohypophyseal DAergic (THDA) neurons and periventricular hypophyseal DAergic (PHDA) neurons from rostral ARN and periventricular nucleus (PeVN) terminating in the neurointermediate lobe of the pituitary gland is less understood. The aim of this study was to assess the role of THDA and PHDA neurons in PRL secretion by measuring DA concentration and characterizing neuronal activity in ARN and PeVN during the diurnal (D) and nocturnal (N) PRL surges of pseudopregnant rats. On the fifth day of pseudopregnancy brains were harvested after decapitation or fixation at 11.00, 13.00, 15.00, 18.00, 21.00 (D surge) and 24.00, 03.00, 06.00, 09.00 (N surge). PeVN's and ARN's of fresh frozen brains were micropunched and DA concentration was mearured by HPLC. The DAergic neuronal activity was assessed by quantitating double label immunocytochemistry for Fos and tyrosine hydroxylase on the fixed brains. Results indicate a decrease in DA concentration accompained by a decrease in neuronal activity of both PeVN and rostral ARN coinciding with the initiation of PRL surges. Middle portions of ARN demonstrated an increase in DA concentration which peaked during the descending phase of PRL secretion. Caudal ARN had a decrease in both DA concentration and neuronal activity during the D PRL peak. These data suggest that changes in DA content of cell populations in PeVN and ARN can be attributed to PHDA, THDA and TIDA neuronal activity. Taken together PHDA, THDA and TIDA neurons appear to have differential roles in modulating the dynamics of PRL secretion. Supported by NIH: DK 43200 and HD 11669.

743.5

EFFECTS OF CENTRALLY ADMINISTERED PROLACTIN ON LHRH CONTENT AND PITUITARY RESPONSE TO LHRH IN DOVES. J.D. Buntin,* J.P. Advis, M.A. Ottinger, R.W. Lea, and P.J. Sharp. Dept. Biol. Sci., U. Wisconsin-Milwaukee, Milwaukee, WI 53201; Dept. Animal Sci., Rutgers U., New Brunswick NJ 08903; Dept. Poultry Sci., U. Maryland, College Park MD 20742; Dept. Applied Biol., U. Central Lancashire, Preston PR1 2HE, U.K.; Div. Develop. & Reprod., Roslin Institute, Midlothian EH25 9PS, U.K.

Intracerebroventricular (ICV) injections of prolactin (PRL) exert potent antigonadal and antigonadotropic effects in ring doves (*Streptopelia risoria*) at doses that are insufficient to stimulate prolactin-dependent crop growth. To explore the physiological basis of these effects, we tested the ability of ICV-injected PRL to influence pituitary responsiveness to luteinizing hormone releasing hormone (LHRH) and to alter LHRH content in the preoptic area (POA) and median eminence (ME). LHRH-induced changes in plasma LH were monitored in photostimulated male doves pretreated for 5 days with ovine PRL (1μg/day, ICV) or saline vehicle (2μl). Although PRL reduced basal plasma LH levels and testes weight, it did not attenuate the plasma LH response to an effective LHRH challenge (0.5μg chicken LHRH/kg, intravenous injection). This suggests that ICV-injected PRL does not suppress LH by reducing pituitary responsiveness to LHRH. In separate studies, changes in LHRH content in the POA and ME were measured by RIA in male doves given 5 days of ICV PRL (1μg/day) or vehicle (2μl) pretreatment and in female doves tested at 1, 6, 12 and 24 h after a single ICV injection of PRL (2μg) or vehicle (2μl). Although reductions in plasma LH were observed following ICV PRL in both studies, no effects of PRL on LHRH content were apparent. Competitive enzyme immunoassay for LHRH also failed to detect differences in median eminence LHRH content between PRL- and vehicle-pretreated male doves. These results suggest that ICV PRL may suppress the gonadal axis by influencing LHRH synthesis, degradation, and/or release; however, integrated measures such as LHRH content do not reflect the specific changes involved. (*Supported by NIMH MH 41447*)

743.6

EFFECTS OF BICUCULLINE ON THE LH RELEASE IN THE PERIPUBERTAL MALE RAT. Dai Mitsushima* and Fukuko Kimura Deptartment of Physiology, Yokohama City University School of Medicine, Yokohama 236, Japan.

In order to examine a role of γ-aminobutylic acid (GABA) in the control of onset of puberty in rats, a GABAA receptor antagonist, bicuculline methiodide, was infused intravenously in prepubertal (16-17 days of age), midpubertal (30-31 days of age) and adult (over 45 days of age) male rats for 30 min. Sequential blood samples (110 μl) were obtained every 15 to 30 min from unanethetized freely moving rats through intraartrial cannula and serum LH concentrations were determined by RIA. Although neither bicuculline nor saline infusion significantly altered LH release in prepubertal stage, in midpubertal stage, 6 of 8 rats were increased in LH release by 20 mg/kg of bicuculline and 5 of 6 rats by 40 mg/kg of bicuculline. The significant responses continued for15 min at 20 mg/kg and 30 min at 40 mg/kg doses. In adult stage, infusion of both doses of bicuculline induced a prompt increase in LH release in all rats. The significant responses continued for 30 min in 20 mg/kg and 45 min in 40 mg/kg. In addition, direct bicuculline injection into the 3rd ventricle (60 ng/μl) also induced a prompt LH release in adult male rats ($p<0.01$). In order to examine whether some testicular factors change the GABAA receptor mediated inhibition, similar experiments were performed in orchidectomized adult rats. Neither 20 mg/kg of bicuculline nor saline infusion induced significant changes in LH release, but 40 mg/kg of bicuculline significantly inhibited the release of LH. It is concluded that, in the male rat, the maturation of the GABA neuronal system to inhibit the release of LH occurs sometime after the onset of puberty and GABA neurons may partly mediate the testicular effect on the control of LH release.

743.7

ANALYSIS OF THE STIMULATING EFFECT OF BICUCULLINE INFUSION ON LH SECRETION IN PROESTROUS RATS. F. Kimura* and K. Jinnai. Department of Physiology, Yokohama City University School of Medicine, Yokohama 236, Japan.

We have shown that an iv infusion of $GABA_A$ receptor antagonist bicuculline (BIC) in the morning produced a premature LH surge in the proestrous rat [Kimura and Jinnai, Horm Behav, 28, 424, 1994]. Present study was performed to further analyze this BIC effect. Female rats of Wistar-Imamichi strain received two intraatrial cannula implantations, one for blood sampling and the other for infusion. BIC ((-)bicuculline methiodide) dissolved in saline was infused iv at a dose of 50 mg/kg/h for 3 h with a peristaltic pump. Pentobarbital sodium (PB) was injected ip at a dose of 32 mg/kg. Serum concentrations of LH were measured by RIA. The result was as follows. 1. On the day of proestrus, BIC infusion between 900-1200 h did not advance the LH surge of LH, but if the infusion was extended to 1300 h, LH surge began at 1200 h. When BIC infusion was started at 1000 h, LH surge began at 1200 h, but If the infusion was started at 1100 h, LH surge began at 1300 h. 2. On the days other than proestrus, BIC infusion between 1000-1300 h did not induce LH secretion at all. 3. PB injection at 1145 h during BIC infusion between 1000-1300 h prevented LH surge which otherwise should have begun at 1200 h. Further, BIC (70 mg/kg/h) infusion between 1400-1700 h, after PB injection at 1400 h, produced LH secretion which began at 1600 h.

The results indicate that, 1) $GABA_A$ receptor system inhibiting the LH surge begins to be relieved from the tension around 1200 h on the day of proestrus but not on the other days, and 2) bicuculline methiodide iv injected acts at the site of barbiturates action to block the surge of LH secretion. Since $GABA_A$ receptors have both the GABA- and barbiturate-binding site, it is concluded that $GABA_A$ receptor system is the major neural substrate involved in the regulation of LHRH surge.

743.8

SURGE GENERATING AND PULSE GENERATING GnRH NEURONS IN RATS. T.FUNABASHI*, K.JINNAI and F.KIMURA. Department of Physiology, Yokohama City Univ. Sch. of Med., Yokohama 236, Japan

In the present study, we examined whether gonadotropin-releasing hormone (GnRH) neurons that expressed Fos in response to the stimulation to the GnRH surge generator were different from those in response to the stimulation to the GnRH pulse generator by double labeling immunocytochemistry (Fos; PC05, Oncogene, GnRH; LRH13 Dr. Wakabayashi).

Rats which had exhibited regular 4-days estrous cycle were used. On the morning of proestrus (08:00-09:00), each rat received an implantation of intraatrial cannula. Naloxone (5 mg/h, iv., 10:00-11:30) or bicuculline (50 mg/kg/h, iv, 10:00-13:00) was infused with a microinfusion pump through the cannula. Control rats received saline infusion. Then, rats were killed by injection with an overdose of pentobarbital sodium (PB, 80mg/kg, iv) at 11:30-12:00 for naloxone treatment and 14:00-14:30 for bicuculline treatment. Some of naloxone-treated rats were pre-treated with PB (32mg/kg, ip).

We found that naloxone treatment, which has been shown to activate GnRH pulse generator, induced Fos within GnRH neurons mainly in the caudal part of the forebrain regardless of PB pre-treatment. On the other hand, bicuculline treatment, which has been shown to advance LH surge, induced Fos within GnRH neurons mainly in the rostral part of the forebrain.

We conclude that GnRH neurons involved in the surge generator are different from those involved in the pulse generator.

743.9

INTERACTIVE EFFECTS OF SOCIAL CUES AND STEROIDS ON GONADOTROPIN-RELEASING HORMONE IMMUNOREACTIVE CELL NUMBERS. E.F. Rissman* and W.J. Cholbi. Department of Biology, University of Virginia, Charlottesville, VA 22903

Interactions with males have rapid and pronounced effects on the numbers of immunoreactive gonadotropin-releasing hormone (GnRH-ir) neurons in female musk shrews. Previously we have reported that contact with a male rapidly (within 15 minutes) increases GnRH-ir cell numbers. Mating and subsequent ovulation have delayed effects on the numbers of GnRH-ir cells (15-40 hours later). Around the time of ovulation, estradiol levels are negatively correlated with GnRH-ir cell numbers. In the present study we examined the hypothesis that ovarian steroids are required to facilitate changes in GnRH-ir cell numbers induced by contact with a male. Females were either ovary-intact or ovariectomized (OVX). One week later, half of the females in each group (n=6-7 per group) resided across a screen barrier from a male for 2 days. Animals were sacrificed, perfused with modified Zamboni's fixative, and tissue sectioned to 30 microns. Every fourth section from each brain was processed for ICC, using a monoclonal antibody (SMI-41) specific for the mature form of GnRH peptide. All GnRH-ir cells were counted from the olfactory bulb through the hypothalamus. A significant interaction between the presence or absence of a male and gonadal state on GnRH-ir cell number was noted in the olfactory bulb, total olfactory related brain areas, and the entire forebrain. Ovary-intact animals that resided for 2 days in contact with a male had significantly more GnRH-ir cells in the forebrain as compared with OVX animals in the same housing condition ($P<0.05$). OVX caused a significant decrease in GnRH-ir cells in the areas mentioned above, and in the tenia tecta. ($P<0.04$ at least). The presence of a male decreased the numbers of GnRH-ir cells in the hippocampus and cortex ($P<0.02$). These data show that steroid hormones facilitate the effect of social cues on the amount of GnRH peptide present in cell bodies. This work is supported by NSF grant IBN 94-12605.

743.10

INFLUENCE OF CONSPECIFIC MALES ON THE GnRH NEURONAL SYSTEM IN NORMAL AND HYPOGONADAL FEMALE MICE WITH PREOPTIC AREA GRAFTS (HPG/POA): AN IMMUNOCYTOCHEMICAL STUDY. G.V. Rajendren* and M.J.Gibson, Dept. Medicine, Mount Sinai Sch. Medicine, New York, NY 10029

Immunocytochemical detection of Fos protein in GnRH neurons of normal and HPG/POA female mice was employed to assess GnRH neuronal activation following exposure to conspecific male stimuli. Normal ovariectomized (OVX) mice were primed with estrogen (E) 48 h prior to testing, and subjected to either 2 h mating or 2 h exposure to male-soiled bedding or clean bedding, with or without progesterone (P) 4-5 h before testing. Robust induction of Fos immunoreactivity was observed in the GnRH neurons following mating in E+P-primed mice; several GnRH neurons in the E-primed females exposed to male-soiled bedding were also Fos+. None of the other treatment groups exhibited Fos immunoreactivity in their GnRH neurons. Plasma LH levels were also elevated in the E+P-primed females subjected to mating and in the E-primed females exposed to male-soiled bedding.

In continuing analyses of Fos immunoreactivity in GnRH cells within grafts in HPG/POA mice, we included several mice which failed to exhibit ovarian development after receiving grafts. Since normal OVX E+P-primed females consistently exhibited Fos immunoreactivity in their GnRH neurons, the HPG/POA females were OVX, primed with E+P and subjected to 2 h mating. In one HPG/POA, whose graft was in the lateral ventricle, there was no Fos immunoreactivity in GnRH neurons. In contrast, many GnRH neurons were Fos+ in the graft of another undeveloped HPG/POA after mating. Most of the graft tissue was located above the anterior commissure with a portion of it extending into the third ventricle. Neither of these females had GnRH innervation of the median eminence or elevated LH release. Thus even when POA grafts are not successful in stimulating ovarian development in HPG females, appropriate neuronal connections for activation of mating-induced Fos expression may be present. The identity of such afferents is under study. HD19077

743.11

EFFECTS OF HEMIOVARIECTOMY (Hovx) ON OVULATION AND MONOAMINE CONCENTRATION IN THE PREOPTIC-ANTERIOR HYPOTHALAMIC AREA (POA-AHA).

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There is evidence that the mechanisms regulating compensatory ovulation and ovarian hypertrophy present asymmetry and lateralization. Also, that Hovx modifies the contents of monoamines in POA-AHA. Then, the effects of left or right hemiovariectomy (Hovx-L; Hovx-R), performed at 12.00-14.00 h of each day of the estrous cycle on ovulation rate and monoamine concentration on POA-AHA was evaluated. The concentration of norepinephrine and dopamine were not modified by Hovx.

When Hovx was performed on diestrus 2, the ovulation rate was reduced significantly (Hovx-L: 2/6; Hovx-R: 1/6 *vs.* control 8/9, $p<0.05$). The concentration of serotonin in those animal with that did not ovulate, presented a different pattern: when the right ovary was extirpated (Hovx-R) the concentration of serotonin was significantly increased (1.524 ± 0.17 ng/mg wet tissue vs. 0.638 ± 0.08, $p< 0.001$), meanwhile when the left ovary was excised, the serotonine concentration in POA-AHA was similar to control group (0.878 ± 0.26 vs, 0.638 ± 0.08). Present results suggest that the lateralized differences observed on the effects of Hovx on ovulation could be related to changes on the serotoninergic levels in POA-AHA.

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743.12

PLASMA PROGESTERONE LEVELS IN PREPUBERTAL RATS WITH GONADOTROPIN STIMULATION AND CATECHOLAMINERGIC BLOCKADE.

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Gonadotropin administration in prepubertal rats induce a increase in follicular growth and steroid secretion mediated by a interelatioship between ovary-hypothalamus-hypophysis axis and diverse systems of neuromodulators. In this work we investigate the participation of catecholaminergic system on progesterone levels and preovulatory follicular growth in 27 days old rats treated with : a) PMSG (8 iu) , b) PMSG, 48 h after Reserpine (RSP:2.5 mg/kg bw), c) PMSG+RSP, 6 h after hCG (10 iu). Animals were sacrified 54 or 72 h after PMSG. Results are presented in the next table:

Group	Ovulation rate	Ova shed	Progesterone (ng/ml)	Preovulatory follicles
54 h				
PMSG	0/5	0 ± 0	19 ± 2	12 ± 1
PMSG+RSP	0/5	0 ± 0	5 ± 0.3 a	6 ± 3
72h				
PMSG	9/9	16 ± 2	17 ± 2	1 ± 0.3
PMSG+RSP	0/5 a	0 ± 0 a	10 ± 1 a	8 ± 2 a

a, $p < 0.05$ vs group PMSG same hour

All animals treated with hCG ovulated (PMSG+hCG: 6/6, ova shed 28 ± 2; PMSG+RSP+hCG: 6/6, ova shed 17 ± 1). Our results suggest a stimulatory participation of central catecholaminergic system on neuroendocrine regulation of gonadotropin secretion.

Supported by DGAPA, PUIS and CONACyT

743.13

BILATERAL ENUCLEATION AT BIRTH DIMINISHES SIMULATED MIDCYCLE GONADOTROPIN SURGES IN ADULT FEMALE RATS.

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In rats the secretion of gonadotrophins is dependent on an intact suprachiasmatic nucleus. Since (1) the circadian secretion of gonadotropins is sexually dimorphic, (2) estrogen is the developmental hormone that regulates the sexual phenotype of the brain nuclei thus far studied and (3) we have demonstrated immunoreactive estrogen synthetase to be present in axons in the optic tract and in central targets of visual afferents including the suprachiasmatic nucleus, we proposed that retinal projections may participate in the development of gonadotrophin control.

Experimental: Newborn female rats were enucleated at birth (n=5), left intact (n=6) or sham operated (n=4). Intact males (n=5) were controls. Three month later all animals were castrated. After 3 weeks a simulated midcycle surge was induced by two daily subcutaneous estradiol injections (10 µg/kg) plus 500 µg progesterone s.c. 24 h later. **Results:** The development of enucleated pups did not differ from controls: vaginal opening occurred between P35-40, followed by 4 day vaginal cycles. Three weeks after castration, LH levels by RIA were elevated in all animals (Σ 16 ng/ml). Blood taken just prior to progesterone showed suppressed LH levels in all cases (Σ 3 ng/ml). Six hours after progesterone, control females' LH was markedly elevated (Σ 15 ng/ml) while enucleated females and control males had no rise (Σ 3 ng/ml). Similar results were obtained when the experiment was repeated in 8 months. **Conclusions:** Despite apparently not impairing the development of ovarian cycles in female rats, neonatal enucleation blocked the induced gonadotrophin surge. In addition to supporting our hypothesis of a developmental role of the visual tract via estrogen formation, these studies indicate that this process may be underway prior to birth. (Support: NIH Grant HD13587 to F.N., Brown-Coxe Fellowship to T.L.H)

743.14

EFFECTS OF GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) ON LHRH AND GONADOTROPIN RELEASE *IN VITRO*.

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Granulocyte-macrophage colony stimulating factor (GM-CSF), a haemapoietic cytokine, is currently reported to play a unique role in gestation. Especially during preimplantation, GM-CSF produced by stimulation of estrogen acts on embryos and results in the success of reproduction. However, no study has been done regarding direct effects of GM-CSF on gonadotropic hormones. Thus, the present study examined *in vitro* whether GM-CSF was implicated in gonadotropin regulation. Hemipituitaries and medial basal hypothalami were dissected from male rat brains and incubated for 2 h and 0.5 h, respectively, in Krebs-Ringer bicarbonate glucose media in a Dubnof metabolic shaker with an atmosphere of 95 % O_2/5 % CO_2. The actions of recombinant murine GM-CSF on pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and luteinizing-hormone releasing hormone (LHRH), were tested *in vitro*, and dose responses of LH and FSH (GM-CSF, 10^{-18} to 10^{-8} M) and LHRH (GM-CSF, 10^{-13} to 10^{-8} M) release were analyzed statistically. At a dose of 10^{-16} M, GM-CSF increased the release of LH ($p < 0.05$) but did not affect FSH release at any doses tested. In contrast, LHRH release was inhibited significantly at doses of 10^{-11}-10^{-12} M, which was evident in a U-shaped dose-dependent manner. The inhibitory effects of GM-CSF (10^{-11} M) on LHRH were reversed by co-incubation with antiGM-CSF (1 µg/ml). The results indicate that GM-CSF acts centrally to inhibit LH release but directly on the pituitary to stimulate LH release. (Supported by MH51853)

743.15

ESTRADIOL-17ß ACUTELY INCREASES POMC mRNA LEVELS AND ß-ENDORPHIN SECRETION FROM THE RAT HYPOTHALAMUS.

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The role of estrogen in the regulation of hypothalamic ß-endorphin neuronal activity was determined *in vitro* and *in vivo*. Using primary cultures of cells from fetal mediobasal hypothalami, we determined the effect of estrogen (estradiol-17ß) on ß-EP precursor POMC mRNA levels and on the ß-EP secretion *in vitro*. Estradiol-17ß dose-dependently increased the levels of ß-EP from the cultured neurons between 3-24 h. However, these cultured neurons developed desensitization to the stimulatory effect of estrogen at 48 h. The steroid also increased cellular levels of POMC mRNA at 24 h. *In vivo*, 24 h following s.c. injection of 10 µg estradiol benzoate, ovariectomized rats showed a significant increase in ß-EP concentrations in pituitary portal blood when compared with those in oil-treated controls. These data suggest that, in addition to known inhibitory effect of estradiol, the steroid may also stimulate ß-EP synthesis and secretion. Supported by National Institutes of Health Grants AA08757 and HD20498.

743.16

EVIDENCE THAT PROGESTERONE INCREASES THE NUMBER OF DELTA OPIOID RECEPTORS IN THE PRE-OPTIC HYPOTHALAMUS OF THE EWE DURING THE LUTEAL PHASE OF THE ESTROUS CYCLE.

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The extent to which endogenous opioid peptides negatively regulate GnRH secretion may vary with physiological status. We have measured the number and affinity of µ, δ and κ subtypes of the opioid receptor in the preoptic area (POA) of the hypothalamus of groups (n=4) of ovariectomised (OVX) ewes that were either untreated or given estrogen (E), progesterone (P), or E & P for 10 days. We also studied ewes (6-8/group) at various stages of the estrous cycle. POA membranes were used for Scatchard analysis for the 3 subtypes of opiate receptor (Shen et al., 1995, J. Endo. In Press).

In OVX ewes, P increased mean (±SE) δ opioid receptor number (172±44 vs 39±9 fmol/mg protein in Controls) with no change in affinity. P and E had no effect on other receptor subtypes. In cycling ewes there were more δ receptors in the luteal phase of the cycle(133±71fmol/mg protein) than in the early- (64±12 fmol/mg), mid- (68±29 fmol/mg) or late- (35±16 fmol/mg) follicular phase.The number and affinity of the µ and κ receptors was similar across the cycle.

The data suggest that the δ sub-type of opioid receptors in the POA is regulated by P and the higher number in the luteal phase of the cycle could account for increased opioid tone at this time.

743.17

IS A DECREASE IN OPIOID TONE NECESSARY FOR THE TIMING OF THE LH SURGE IN RATS? P.B. Lieberman, C.A. Paronis, J.H. Woods, E.A. Young*. Departments of Physiology, Pharmacology, and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Based upon previous opioid agonist and antagonist studies it has been hypothesized that a decrease in opioid tone at the mu receptor subtype is the necessary signal to trigger the preovulatory LH surge in rats. To test this we have used an irreversible mu receptor antagonist, clocinnamox (CCam; 5-10 mg/kg), that has been shown to alkylate receptors and completely abolished morphine analgesia as assessed by tail flick latency for at least 1 week. If a decrease in opioid tone at the mu receptor is the critical signal to trigger an LH surge, we hypothesized that this treatment would interfere with the timing of the LH surge. Briefly, rats underwent daily vaginal smearing for a 2 week baseline period and throughout the study, and were injected with vehicle on the afternoon of diestrus II. Blood samples were collected by tailnick in the morning (0830) and at 1 of the following PM timepoints (1200, 1430, 1530, 1630). After a 1 week recovery period, CCam was administered on diestrus II and blood sampling was repeated. CCam caused a significant increase in plasma LH levels at all timepoints except during the surge. In most rats, the surge was initiated between 1430 and 1530, although two rats had reached surge LH levels by 1430 in both their control and C-Cam treated cycles. These data suggest that although tonic LH secretion is inhibited by activity at the mu receptor, it is not the critical trigger for the LH surge. (Supported by MH 45232 and DA 00254 and DA 05653.)

743.18

DIRECT EVIDENCE FOR DOWN-REGULATION OF HUMAN GONADOTROPIN- RELEASING HORMONE (GnRH) GENE BY ESTROGEN K.W. Dong[1]* Z.G. Chen[1], K.W. Cheng[2] and K.L. Yu[2]. 1The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA 23507. 2Department of Zoology, The University of Hong Kong, Hong Kong.

Although expression of the human GnRH gene has been reported in hypothalamus and reproductive tissues, the molecular mechanisms underlying the regulation of GnRH gene expression by estrogen in these tissues are largely unclear. The apparent discrepancies among various *in vivo* studies may be due to the complex neuronal circuits and the lack of estrogen receptors in certain populations of GnRH neurons. This study seeks to determine whether estrogen could have a direct effect on human GnRH gene expression in transfected placental tumor cells (JEG). Our previous study identified two promoters in the human GnRH gene with the upstream promoter being used only in reproductive tissues such as placenta . In this study, the downstream GnRH promoter (H-1) containing 0.65 kb of the 5' flanking sequence and the 1.8 kb of the upstream promoter region (H-2) with a deletion of the downstream promoter sequence, were fused to a promoterless luciferase reporter construct. The response of these promoter-luciferase constructs to estrogen was assessed by transfection into the JEG cells. The luciferase activity in the transfected cells was measured after either no treatment or treatment with various concentrations of estradiol, JEG cells transfected with H-1-Luc or H-2-Luc construct showed insignificant changes in luciferase activity in response to estradiol. However, cotransfection of H-1-Luc or H-2-Luc construct into JEG cells with a vector expressing a human estrogen receptor (ER)cDNA resulted in decreases in luciferase activity in response to estradiol. Thus, this study demonstrated that both upstream and downstream human GnRH promoter regions can confer down regulation by estrogen in transfected JEG cells in the presence of cotransfected ER expression vector.

743.19

CONSISTENT WITH REMOVAL OF LOCAL INHIBITION, PERIFUSION RATE AFFECTS LH AND FSH SECRETION. **Lemon, WJ, Padmanabhan, V, Favreau, PA, Heinze, K and Midgley, AR*, Reproductive Sciences Program, Cellular Biotechnology Training Program, National Center for Infertility Research, Bioengineering Program, University of Michigan, Ann Arbor, MI 48109-0404.**

In addition to hypothalamic GnRH, local pituitary factors (e.g. inhibin, activin, galanin) play an important modulatory role in regulating secretion of LH and FSH. Since GnRH binding to receptor depends primarily on its concentration, secretion that depends solely on GnRH should occur independently of perifusion rate while secretion that depends on local factors will change as the rate changes. Anterior pituitary cells were obtained from abattoir sheep, dispersed, co-loaded with beads and perifused in a custom, computer-controlled micro-perifusion system from which LH and FSH were measured. Four groups of cells were acclimatized in the chambers for 19 h without GnRH stimulation, then stimulated every 40 min for 8 h with 4 min, 1.0nM pulses of GnRH. The first group was perifused at a constant high flow rate (136μL/min.); the second group at a constant low flow rate (14μL/min.); the third group was switched from the high rate to the low rate (4h/4h); the fourth group was switched from the low rate to the high rate (4h/4h). Secretion (ng/5') decreased when the flow decreased (ratio of means of log-transformed data 4-8h/0-4h for LH=0.79; FSH = 0.85). Release of both gonadotropins increased when the flow rate increased (ratios: LH=1.12; FSH=1.24). At constant flow rate, LH decreased over time (ratios: high flow rate=0.93; low flow rate=0.91) but FSH remained constant (ratio=1.00 at both high and low flow rates). These data are consistent with removal of common local inhibition, but other models cannot be ruled out. Supported by NIH grants U54 HD 29184 & P30 HD 18258.

743.20

EFFECT OF PROGESTERONE ON LHRH, LHRH RECEPTOR AND LHβ mRNA LEVELS IN OVARIECTOMIZED AND ESTROGEN-TREATED RATS: AN INSIGHT INTO THE MECHANISM OF LHRH-LH SURGE BY STEROIDS. J. Y. Seong, S. S. Kang, K. Kam, W. Sun and K. Kim.* Dept. of Molecular Biology, Col. of Nat. Sci. Seoul Nat'l Univ., Seoul 151-742, Korea.

The present study is designed to evaluate the stimulatory action of progesterone (P) on the LHRH-LH axis. Using ovariectomized and estrogen (OVX+E)-treated rats, we determined LHRH mRNA level in the preoptic area (POA), and LHRH receptor (LHRHR) and LHβ mRNA levels in the pituitary by competitive RT-PCR or Northern blot analysis. Silastic capsule containing 17β-estradiol (E, 180 μg/ml in oil: 30 mm in length, id 1.575 mm) was implanted to OVX rat, and 2 days later a single injection of P was administered s.c. at 10:00 h. P increased serum LH levels in a dose-dependent manner and 1 mg of P was enough to enhance the maximal increase in serum LH level at 17:00 h. At this time, LHRH mRNA level in the POA was also augmented by P in a dose-dependent manner. In the pituitary, LHRHR mRNA level was increased by P, while there was no significant change in LHβ mRNA level. To determine the temporal changes in such parameters, several time points were chosen according to the profile of serum LH levels. LHRHR mRNA level was increased 2 hr prior to LH surge (at 17:00 h) and declined when serum LH level was decreased. LHRH mRNA level rised at the time of the LH surge and the elevated level was sustained until 22:00 h. No change in LHβ mRNA level, however, was observed. These results show that 1) LHRHR mRNA level in the pituitary was elevated prior to the LH surge, which may be involved in the potentiation of LH surge, and 2) an increase in LHRH mRNA level in the POA is accompanied by the LH surge, and sustained elevated LHRH mRNA level may be related to the desensitization of LHRHR and consequent decrease in LH levels. Taken together, the present study indicates that P may play an important role in regulating LHRH and LHRHR gene expression at the time of the LH surge.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION VI

744.1

INFLUENCE OF LIFE-LONG MODERATE CALORIC RESTRICTION ON NEUROPEPTIDE-Y GENE EXPRESSION IN THE ARCUATE NUCLEUS OF FEMALE RATS. **T.M. McShane* and P.M. Wise.** *Department of Physiology, University of Kentucky, College of Medicine, Lexington, KY 40536-0084.*

Moderate caloric restriction extends lifespan, reduces the incidence of age-related diseases, and delays reproductive senescence in rats. We have previously shown that life-long moderate caloric restriction delays onset of persistent estrus and enhances pulsatile secretion of LH. Neuropeptide-Y (NPY) is thought to play an important role in maintenance of reproductive cycles, feeding behavior, and metabolic homeostasis. We hypothesized that moderate caloric restriction (CR) works through NPY-mediated mechanisms to delay reproductive aging. Female Sprague-Dawley rats (7 weeks old) were placed on caloric restriction (CR; n=70) which was 60% of ad libitum (AL) intake measured in control rats (n=70). Rats were individually housed under a 14L:10D cycle, and fed daily within 1.5 hours of lights-off. Rats were rapidly decapitated 2.5 weeks following OVX, when rats were 4, 12, or 18 months of age. Brains were dissected, frozen, and coronal sections (12 microns) through the arcuate nucleus were cut at -20°C on a cryostat. Relative levels of NPY mRNA were measured by In situ hybridization. A cDNA clone complementary to rat prepro-NPY was used as a transcription template to synthesize a cRNA probe labeled with [^{35}S]UTP. Slides were dipped in photographic emulsion, exposed for 10 d before developed, and silver grains were quantified using computer assisted image analysis. Number of NPY cells per section in the anterior and mid-arcuate was not different between CR and AL rats and did not change with age. Relative level of NPY mRNA (area of pixels covered by silver grains) was greater in CR rats ($P<.001$), but was not influenced by age or previous cycle history. Increased biosynthesis of NPY may contribute to the enhanced amplitude of LH pulses previously reported in these animals. Supported by NIH AG02224 to PMW and AG05648 to TMM.

744.2

SODIUM PENTOBARBITONE AND THE INHIBITION OF LUTEINIZING HORMONE PULSES IN RATS: THE SIGNIFICANCE OF HYPOTHERMIA. P. H. Strutton and C. W. Coen* Division of Biomedical Sciences, King's College, London WC2R 2LS, U.K.

The role of hypothermia in the suppression of pulsatile luteinizing hormone (LH) release by the general anaesthetic sodium pentobarbitone has been investigated in ovariectomised rats. Each animal was fitted with an intraperitoneal miniature radio transmitter to monitor core temperature and with an indwelling cannula in the right atrium of the heart and in the intraperitoneal space. During the 6-hour sampling period the animal's core temperature was recorded automatically every 5 minutes and a 25 μl blood sample was obtained concurrently using an automated system. After the initial 3 hours of sampling either the drug or the vehicle was administered via the intraperitoneal cannula from outside the cage ensuring minimal disturbance to the animal. Administration of sodium pentobarbitone (40 mg/kg) at an ambient temperature of 21°C resulted in a significant hypothermia throughout the 3-hour post-injection period. During this period there was a significant reduction in mean LH concentration, pulse amplitude and pulse frequency. When the drug was administered at an ambient temperature of 35°C there was no significant reduction in core temperature and no significant change in the LH pulse parameters. Administration of the vehicle had no significant effect on core temperature or on the LH pulse parameters at either 21°C or 35°C. These results indicate that the effects of this barbiturate on the pulsatile release of LH are secondary to the induced hypothermia. We are investigating the extent to which the induction of hypothermia may be of critical importance in the suppression of LH pulses by other drugs.

744.3

VOLUNTARY EXERCISE INHIBITS BASAL GONADOTROPIN SECRETION IN OVARIECTOMIZED, NON-REPLACED, BUT NOT OVARIECTOMIZED HAMSTERS TREATED WITH ESTRADIOL.

David Pieper*, Haythem Ali, Subhashini Ladella, Catherine Lobocki, and Katarina Borer, Providence Hospital, Department of Physiology, Southfield, Michigan, and the University of Michigan, Department of Kinesiology, Ann Arbor, Michigan

Recent studies in male hamsters have shown that voluntary exercise facilitates gonadotropin secretion by inhibiting the negative feedback of testosterone. The present study investigated the influence of exercise on basal (morning) gonadotropin levels in ovariectomized hamsters treated with blank capsules or with capsules containing estradiol.

Forty-eight adult female hamsters were ovariectomized and implanted with capsules which were either blank or contained 4 mm of estradiol 17B (E2). One half of each E2 group was placed in cages with wheels (EX group) or in similar cages without wheels (SED). Blood samples were obtained between 8 and 10 AM by retro-orbital puncture before surgery and at weekly intervals following surgery for 5 weeks.

There was a dramatic increase in serum LH and FSH following ovariectomy in both groups without E2, but all E2 treated hamsters had low levels throughout the study. There was no consistent difference in LH or FSH levels between the SED and EX groups with E2, but the serum FSH (and to a lesser extent LH) was consistently significantly lower in the EX than SED groups with blank capsules.

We conclude that exercise has a steroid independent effect to inhibit basal gonadotropin secretion in female hamsters.

744.4

EFFECT OF FOOD DEPRIVATION ON ESTROGEN RECEPTOR IMMUNOREACTIVITY IN DIFFERENT HYPOTHALAMIC NUCLEI OF FEMALE SYRIAN HAMSTERS. A. K. Panicker, H-Y Li, J.D.Blaustein and G.N. Wade*. Neuroscience and Behavior program, University of Massachusetts, Amherst, MA01003.

Metabolic fuels have a profound effect on reproductive functions of female Syrian hamsters. Estradiol plays an important role in the physiological and behavioral components of reproduction, ovulation and estrous behavior. Mapping of neural sites where estrogen receptor immunoreactive (ERIR) cells respond to metabolic fuels and the type of response might enable us to find a relation between specific sites and behavioral and neuroendocrine events leading to nutritional infertility. Previous research from this lab showed that 48h of food deprivation (FD) in female Syrian hamsters caused a decrease in the number of ERIR cells in the ventromedial hypothalamus, an increase in the medial preoptic area and no effect in the nucleus of solitary tract. In the present study we looked at ERIR cells in the parvocellular portion of the paraventricular nucleus (pPVN), medial amygdala (MeA) and arcuate nucleus (Arc). The number of ERIR cells in the pPVN increased following 48 h of FD. This is in agreement with the hypothesis that the increased release of corticotropin releasing hormone in acute fasting is facilitated by estrogen action in the PVN. Even though MeA is believed to be involved in female sexual behavior, no significant effects on ERIR cells were observed in this region, consistent with the idea that it has little effect on reproduction related to nutrition. In the Arc FD caused a decrease in the number of ERIR cells, which may contribute to the blockade of preovulatory luteinizing hormone surge in food- deprived animals by neuropeptide Y.

744.5

SUPPRESSION OF LH PULSES BY REDUCING GLUCOSE AVAILABILITY IS MEDIATED BY SENSORS IN THE LOWER BRAIN STEM. S. Nagatani[1], K. Murahashi[1], D. C. Bucholtz[2], S. Tsukahara[1], M. A. C. Estacio[1], H. Tsukamura[1], D. Foster[2]* and K.-I. Maeda[1]. [1]Sch. of Agri. Sci., Nagoya Univ., Nagoya 464-01, Japan; [2]Reprod. Sci. Progr., Univ. Michigan, Ann Arbor MI 48109, USA.

Glucose may play a key role in controlling reproductive activity through modulation of GnRH secretion. The present study in the rat determined 1) if glucoprivic suppression of pulsatile LH release is sexually differentiated; 2) if the glucoprivic suppression is potentiated by gonadal steroids; 3) if such glucoprivic suppression of LH secretion is mediated by a glucosensor in the 4th ventricle. Our approach was to monitor pulsatile LH secretion after peripheral (jugular) or central (4th ventricle, 4V) administration of the competitive inhibitor of glycolysis, 2-deoxyglucose (2DG). Fourteen days after gonadectomy, blood samples for LH were collected every 6 min for 3 h. After one hour of sampling, 2DG was administered peripherally (200, 400 or 800 mg/kg BW) in gonadectomized (GDX) males and females in the presence or absence of sex steroids (testosterone or estradiol) or centrally (4 or 40 mg/kg) in GDX or testosterone-treated GDX males. In the presence of steroids, mean plasma LH decreased ($P<0.05$) at all peripheral doses of 2DG, whereas in the absence of sex steroids, the lowest dose was ineffective. The middle dose of 2DG suppressed ($P<0.05$) mean plasma LH in GDX females but not in GDX males. Central 2-DG infusion of 40 mg/kg, but not 4 mg/kg, suppressed pulsatile LH secretion in both GDX and testosterone-treated GDX rats. LH secretion was not depressed by an isosmotic xylose infusion. The site-specificity of the 2DG infusion into the 4V was confirmed by an isovolumetric infusion of dye at the termination of sampling. Collectively, our findings suggest that (1) the hypothalamo-hypophyseal axis of female rats is more sensitive to the decreased glucose availability induced by 2DG than that of males; (2) this glucoprivic suppression of LH pulses is potentiated by gonadal steroids; (3) glucose availability is detected in the 4th ventricle consistent with the notion that the area postrema may serve as an important glucosensor.

744.6

LUTEINIZING HORMONE SECRETION IN RHESUS MONKEYS FOLLOWING CORTISOL SYNTHESIS INHIBITION WITH METYRAPONE. D. A. Van Vugt*, J. Piercy, A. E. Farley, and R. L. Reid, Obstetrics & Gynecology and Physiology, Queen's University, Kingston, Ontario K7L 3N6.

Luteinizing Hormone (LH) secretion can be inhibited by Corticotrophin Releasing Hormone (CRH), and various stressors which increase CRH secretion cause inhibition of LH secretion. In an attempt to further elucidate the physiological significance of CRH regulation of LH secretion we determined the effects of metyrapone on LH secretion. Metyrapone has been shown to activate CRH neurons, presumably as a consequence of reduced cortisol negative feedback. We reasoned that this paradigm could be exploited to study CRH regulation of LH secretion in the monkey. Six ovariectomized rhesus monkeys were placed into primate chairs and angiocatheters were introduced into the femoral and saphenous veins for blood collection and metyrapone infusion respectively. Metyrapone (5 mg/kg/hr) or saline was infused for ten hours from 2300 to 0900 hours. Blood samples for LH and cortisol were collected at 15 minute intervals for six hours starting at 0900 hours.

Metyrapone infusion resulted in a pronounced inhibition of cortisol. The mean ($\pm$SEM) cortisol level at the end of a 10 hour infusion of metyrapone was 6.9 $\pm$ 0.6 μg/dL compared to 46.8 $\pm$ 3.9 μg/dL in saline controls. Cortisol levels gradually increased to 22.2 $\pm$ 3.5 μg/dL during the subsequent six hour blood sampling protocol compared to 53.1 $\pm$ 5.4 μg/dL in the control group. M ean LH levels were 73.4 $\pm$ 6.0 ng/mL following metryapone compared to 75.5 $\pm$ 6.1 ng/mL in saline controls. No effect of metyrapone infusion on pulsatile LH secretion was seen. These results indicate that inhibition of LH secretion does not always accompany activation of CRH neurons. The presence of CRH populations that regulate the pituitary - adrenal axis specifically may explain these findings. Alternatively, CRH may not be a critical neuromodulator of LH secretion in the ovariectomized monkey (This work was supported by the MRC of Canada).

744.7

GLUCOCORTICOID REPRESSION OF GNRH GENE EXPRESSION AND SECRETION IN MORPHOLOGICALLY DISTINCT SUBPOPULATIONS OF GT1-7 CELLS. B. Attardi*, T. Tsujii1, R. Friedman1, Z.W.Zheng2, J.L. Roberts2, T. Dellovade3, D. Pfaff3, and D.B.DeFranco4. *1Med. and 4Biol. Sci., Univ. of Pittsburgh, Pgh., PA 15213; 2Mt. Sinai Med. Ctr., N.Y., NY 10029; 3Rockefeller Univ., N.Y., NY 10021.

GT1-7 cells possess functional glucocorticoid receptors, and the synthetic glucocorticoid, dexamethasone (dex), represses GnRH gene expression (Endo. 134:1467, 1994). We have now compared the dex sensitivity of two variants of GT1-7 cells with different morphological properties (type I: "neuronal" appearance, phase-bright, many processes; type II: phase-dark, fewer processes). Dex (1 μM, 20-24 h) suppressed luciferase activity in transfections with GnRH-luciferase reporter plasmids as well as GnRH mRNA to a greater extent in type II cells than in type I. ICC for GnRH demonstrated dark reaction product uniformly among type I cells, whereas type II cells had little or no stain. For secretion studies, cells were attached to Cytodex beads and perifused for 8 h with control or dex-containing medium. The high levels of GnRH secreted by type I cells were slightly enhanced by dex. In type II cells, which secreted much lower levels of GnRH, dex decreased GnRH release rapidly and profoundly. Thus, in the type II subpopulation of GT1-7 cells, nuclear alterations leading to greater transcriptional susceptibility to dex, coupled with low GnRH storage levels, may be reflected in exquisite sensitivity of GnRH secretion to glucocorticoid repression.

744.8

CORTICOTROPIN RELEASING HORMONE ANTAGONIST BLOCKS Δ^9-TETRAHYDROCANNABINOL-INDUCED SUPPRESSION OF LUTEINIZING HORMONE SECRETION IN OVARIECTOMIZED RATS. A.L. Jackson and L.L. Murphy *. Dept. of Physiology, Southern Illinois Univ. Sch. of Med., Carbondale, IL 62901.

Findings that marijuana and Δ^9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, stimulate the hypothalamic-pituitary-adrenal and inhibit the -gonadal axes are well documented in experimental animals. Recent studies have demonstrated that corticotropin releasing hormone (CRH) can suppress gonadotropin-releasing hormone (GnRH) secretion. Thus, this study was designed to determine if THC inhibits luteinizing hormone (LH) release in ovariectomized (OVX) rats, via a CRH-mediated mechanism. Adult rats were OVX and 3 weeks later implanted with stainless steel cannulae within the third ventricle of the brain. One week after brain surgery, polyethylene cannulae were inserted into the jugular vein for THC administration and blood withdrawal. After a 24 h recovery period, the CRH receptor antagonist, α-helical CRF_{9-41} (αh-CRF; 100μg/5μl), or vehicle was infused into the third ventricle (2.5μl/min) after an initial blood sample. THC (0.5 mg/kg b.wt.) or vehicle was administered 30 min after αh-CRF and blood samples were taken at times 30 and 60 post-THC administration. Results revealed that THC produced significant decreases in plasma LH levels at 30 and 60 min post-treatment when compared to vehicle ($p<0.05$). Infusion of αh-CRF did not alter LH levels at any time point relative to its vehicle. However, pre-treatment with the antagonist completely blocked the suppression of LH by THC when compared to animals receiving THC vehicle. These results suggest that CRH mediates the inhibition of LH secretion following acute THC treatment in OVX rats. (Supported by DA-05042)

744.9

CROSS-CORRELATION ANALYSIS OF INTERACTIONS BETWEEN HYPOTHALAMIC UNITS ASSOCIATED WITH THE GONADOTROPIN-RELEASING HORMONE (GnRH) PULSE GENERATOR. T. Ördög and E. Knobil*. Laboratory for Neuroendocrinology, Medical School, The University of Texas Health Science Center, Houston, TX 77225.

The electrophysiological correlates of the activity of the GnRH pulse generator, a hypothalamic oscillator that governs the episodic secretion of the pituitary gonadotropic hormones, are abrupt, rhythmic increases in multiunit activity (MUA volleys) in the mediobasal hypothalamus (MBH) that represent the simultaneous increase in firing rate of individual units. This synchrony could be effected by parallel inputs from a "master oscillator" or by other mechanisms. Single units were identified by cluster analysis of multiunit recordings from the MBH of 3 ovariectomized rhesus monkeys and their connectivity analyzed by cross-correlation histograms of time series of their action potentials, a method that determines the probability that the firing of two neurons relative to one another is not due to chance. To relate the results to the generation of the MUA volleys, the analyses were performed separately during the volleys and the intervals between them. Significant ($p<0.01$) interaction was found in ~60% of the 31 pairs of pulse-generator-associated units analyzed. The majority of cross-correlation histograms consisted of a single peak with a delay from time zero of 1-3 ms and a duration of 0.5-1.5 ms between and during volleys. The distinct character and short duration of this peak suggest, in agreement with observations made by others in the cerebral cortex, direct, synaptic, excitatory interactions between these units. Although the specific characteristics of these interactions (*strength* and *contribution*) varied considerably from one unit pair to another, they were not significantly different during and between volleys. The results suggest that the synchronization of the single-unit bursts may be effected by these direct synaptic interactions rather than by a common Zeitgeber. (Supported by NIH grants HD-17438 and HD-08610, and by the Ellwood Foundation.)

744.10

MODELING THE AGONIST-INDUCED ELECTRICAL AND CALCIUM ACTIVITY PATTERNS OF PITUITARY GONADOTROPHS
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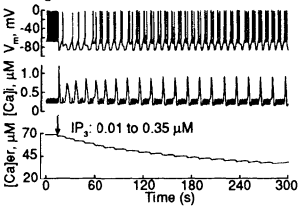
Pituitary gonadotrophs are ideal for studying Ca^{2+} signaling in excitable cells. Signals can be generated separately or interactively via plasma-membrane Ca^{2+} entry through voltage-gated Ca^{2+} channels and/or via hormone-induced Ca^{2+} release from the intracellular Ca^{2+} store, endoplasmic reticulum (ER), through inositol trisphosphate (IP_3) receptor Ca^{2+} channels on the ER membrane. We account for the complex Ca^{2+} and V_m dynamic patterns using mathematical models in combination with Ca^{2+} fluorescence and whole-cell clamp electrical measurements. Our model incorporates Ca^{2+} diffusion and the IP_3-mediated Ca^{2+} oscillation mechanism spatially-distributed in a spherically symmetric cytoplasm with a boundary condition describing the activity of plasma membrane channels and active Ca^{2+} pumps. Besides explaining various firing patterns observed in both male and female gonadotrophs during IP_3-induced Ca^{2+} oscillations, our modeling gives insights into the mechanisms of Ca^{2+} homeostasis before, during, and after agonist stimulation. The Ca^{2+} handling parameters (diffusion rates, buffering capacities, membrane pump and leakage characteristics) all influence the spatio-temporal patterns of Ca^{2+} and V_m. Quantitative understanding of resetting the Ca^{2+} oscillation's phase with current pulses is also achieved.



744.11

A NEURAL NETWORK MODEL OF THE LHRH PULSE GENERATOR: SYNCHRONY AND PULSATILITY. M. D. Loose*, Neuroscience Program, Oberlin College, Oberlin, OH 44074

The secretion of LHRH has a distinctive, pulsatile pattern of short periods of relatively high levels of release followed by longer intervals of low to undetectable levels of release that is thought to result from synchronized release by tens/hundreds of LHRH neurons. To examine how certain variables affect synchronization, I have constructed and tested a simple model consisting of 100 randomly connected LHRH neurodes. All neurodes projected to the median eminence, were endogenous bursters with randomly assigned interburst-intervals (IBI), and had excitatory effects on postsynaptic cells. Each neurode multiplies its inputs by the associated synaptic weights and sums these values. Neurode output was a sigmoid function of the sum and circuit output was the summed neurode output. This process was repeated every sec for 4-8 simulated hours. With 0% connectivity, circuits had only small fluctuations in output over time. If all cells were "stimulated" to burst simultaneously, subsequent pulses decreased in amplitude and increased in duration at a rate dependent on the variability of the IBI. When the chance of a synapse from one neurode to another was set at 1, 2, 3, 5 or 10% and various synaptic weights were tested, circuits were capable of indefinitely maintaining a synchronized output and of creating a pulsatile output from a non-synchronized starting point. "Ablation" of neurodes indicated that pulsatility was a robust feature of circuits exhibiting pulsatile output. This model has numerous variables that require further evaluation but appears to be a useful paradigm for modeling LHRH pulsatility.

744.12

ACTIVATION OF PKA: A NOVEL TRANSDUCTION MECHANISM FOR ESTROGEN'S MODULATION OF μ-OPIOID/K^+ CHANNEL COUPLING. A.H. Lagrange*, M.J. Kelly Dept. of Physiology, Oregon Health Sciences U., Portland, OR 97201

Control of the female HPG axis by estradiol (E_2) involves inhibition of hypothalamic GnRH release via modulation of the β-endorphin system. μ-opioid peptides (e.g. β-endorphin) hyperpolarize hypothalamic cells by opening a K^+ inward-rectifier ($I_{K(ir)}$) Intracellular recordings in arcuate neurons from *in vitro* hypothalamic slices from ovariectomized guinea pigs were used to study the mechanism of E_2 action. The potency of the response to the μ-opioid agonist, DAMGO is rapidly decreased nearly four-fold by a brief exposure to E_2 (100 nM, 20 min) in about 35% of neurons tested. The effect of E_2 is stereoisomer-specific and concentration-dependent. μ-receptor/G-protein coupling is altered by cAMP-dependent protein kinase (PKA), and there is evidence that the estrogen receptor interacts with PKA in some systems. The effects of E_2 were blocked by the nonspecific protein kinase inhibitor, staurosporine (100 nM, n=10), and mimicked by stimulation of adenylate cyclase with forskolin (1 - 20 μM, n=6). The more selective PKA activator, S_p-cAMP mimicked the effects of E_2 in a concentration-dependent manner (n=3). Although the selective PKA antagonists, KT5720 (50 nM, n=7) and R_p-cAMP (100 μM, n=7) inhibit PKA by different mechanisms, both agents blocked the effects of E_2. The present work provides a novel transduction mechanism for E_2 (i.e. PKA activation), as well as elucidating the intracellular mechanisms regulating the actions of opioid peptides. (Supported by PHS Grants DA05158 and MH10327)

744.13

PHARMACOLOGICAL CHARACTERIZATION OF THE ESTROGEN RECEPTOR'S RAPID ALTERATION OF μ-OPIOID SYNAPTIC TRANSMISSION. M.J. Kelly*, A.H. Lagrange Dept. of Physiology, Oregon Health Sciences U., Portland, OR 97201

We have previously shown a rapid effect of 17β-Estradiol (E_2) to alter the potency of the μ-opioid agonist, DAMGO, in hypothalamic neurons. This effect is faster than would be expected for the classic genomic model of E_2 action, and appears to involve activation of PKA. To study the mechanism of E_2 action, intracellular recordings were made from arcuate neurons from *in vitro* hypothalamic slices from ovariectomized guinea pigs. DAMGO hyperpolarizes these cells by opening an inwardly rectifying K^+ channel. The potency of this effect is reduced nearly four-fold by a brief exposure to E_2 (100 nM, 20 min) in about 35% of neurons tested. The EC_{50} for E_2's effect was 8 nM, with a Hill slope of 0.7. The biologically inactive isomer, 17α-E_2 was unable to mimic the effects of 17β-E_2. The effects of E_2 appear to be mediated by an intracellular receptor, as the membrane-impermeant conjugate BSA-E_2 did not alter DAMGO potency. Furthermore, the effects of E_2 were blocked by the "pure" antiestrogen ICI 164,384 and the structurally dissimilar estrogenic drug, diethylstilbestrol. By using an antagonist to shift the E_2 concentration-response curve, Schild analysis resulted in an estimated K_e for ICI 164, 384 of 0.3 nM, similar to the K_i for ICI 164,384 binding to the classical estrogen receptor. Therefore, characteristics of the receptor mediating this rapid estrogen effect are in many ways similar to the classical estrogen receptor and this work may represent a novel signal transduction mechanism for this protein. (Supported by PHS Grants DA05158 & MH10327)

744.14

ENDOTOXIN SUPPRESSES GONADOTROPIN-RELEASING HORMONE PULSE GENERATOR ACTIVITY THROUGH TUMOR NECROSIS FACTOR-α. M.J. Yoo, M. Nishihara*, and M. Takahashi. Department of Veterinary Physiology, Veterinary Medical Science, The University of Tokyo, Tokyo 113, Japan.

Bacterial endotoxin lipopolysaccharide (LPS) can induce a variety of immune responses including a release of tumor necrosis factor (TNF)-α into peripheral circulation and suppress gonadotropin secretion as well. We examined in the rat whether LPS could affect the electrical activity of the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator, which governs pulsatile secretion of gonadotropin from the pituitary, and the involvement of TNF-α, if any, in this process. Ovariectomized rats were fitted with chronically implanted electrode arrays in the medial basal hypothalamus, and multiple unit activity (MUA) was monitored from freely moving animals. By intravenous (IV) injection of LPS (1 μg), MUA volleys and associated luteinizing hormone (LH) pulses were suppressed for several hours. The suppressive effect due to LPS was nullified by the antibody against TNF-α administered intracerebroventricularly (ICV). Graded doses of TNF-α administered either IV (0.4, 1 and 2 μg) or ICV (20, 50 and 250 ng) suppressed both MUA volleys and LH pulses in a dose dependent manner similarly as LPS did. On the other hand, neither IV nor ICV injection of TNF-α affected MUA volleys if animals were pretreated with indomethacin (IV, 1 μg/100 g BW), a cycloxygenase inhibitor, 10 min before. These results suggest that LPS leads to a decrease in hypothalamic GnRH pulse generator activity through TNF-α, and that the suppressive effect of either peripherally or centrally derived TNF-α on the pulse generator depends on the prostaglandins synthesis.

745.1

EFFECTS OF MICROIMPLANTS OF AN ANTIANDROGEN INTO THE ROSTRAL HYPOTHALAMUS ON GABAergic NEURONS AND ON LH SECRETION IN THE INTACT MALE RAT. D.R. Grattan, M.S. Rocca, M.M. McCarthy and M. Selmanoff*. Center for Studies in Reproduction, Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201.

GABAergic neurons terminating in the rostral hypothalamus are stimulated by testosterone. To investigate whether this action is mediated locally through androgen receptors in the rostral hypothalamus, bilateral microcannulae (28 gauge) containing the androgen receptor antagonist, hydroxyflutamide (HF), were stereotaxically implanted into the rostral preoptic region just dorsal to the major population of GnRH cell bodies. Two days later, blood samples were collected for LH assay and animals were sacrificed for determination of GABAergic neuronal activity in tissue microdissected from the site of the implanted cannulae. Animals were decapitated either without treatment, or 60 minutes after inhibition of GABA degradation by AOAA (100 mg/kg, ip). The rate of GABA accumulation in the tissue after AOAA is a measure of GABA turnover. Levels of mRNA for both forms of glutamic acid decarboxylase (GAD_{65} and GAD_{67}), the rate limiting enzyme responsible for GABA synthesis, also were measured by a microlysate RNase protection assay.

LH levels were significantly increased in HF-treated animals (0.29 ± 0.05 ng/ml) compared with controls (0.15 ± 0.02 ng/ml). In the brain region directly ventral to the microcannula tip (500 µm punch from 300 µm fresh frozen section), GABA turnover was significantly reduced in HF-treated rats. There was no effect of HF on either GAD_{65} or GAD_{67} mRNA levels in this brain region. The results indicate that GABAergic neurons terminating in the rostral hypothalamus are tonically stimulated by testosterone acting at an androgen receptor localized in this region. The findings support the working hypothesis that these androgen-sensitive GABAergic neurons mediate the negative feedback action of testosterone on GnRH secretion in the male rat. (Supported by NIH grant HD21351 awarded to MS).

745.2

TESTOSTERONE-INDUCED ACTIVATION OF TYROSINE HYDROXYLASE-CONTAINING NEURONS OF THE A14 AND A15 HYPOTHALAMIC NUCLEI IN THE MALE SHEEP. Lubbers LS[1], Hileman SM[1], Jansen HT[2], Lehman MN[2] and Jackson GL[1]*. [1]Dept. of Veterinary Biosciences, University of Illinois, Urbana-Champaign, Il 61801, [2]Dept. of Anatomy and Cell Biology, University of Cincinnati Medical Center, Cincinnati OH, 45267.

Tyrosine hydroxylase (TH)-containing neurons of A14 and A15 hypothalamic nuclei, which are dopaminergic in nature, appear to mediate the inhibitory effects of gonadal steroids on luteinizing hormone (LH) release in female sheep. However, the role of these neurons in steroid-induced inhibition of LH release in the male is unknown. We tested the hypothesis that in the male TH neurons of the A14 and A15 are activated by testosterone (T). We examined the percent of TH cells which express cFos, a marker of neuronal activation, in castrated male sheep infused with vehicle (n=4) or T (768 µg/kg/d T; n=4) for 72 h. Blood samples were collected every 10 min for 4 h just prior to, and during the final 4 h of infusion. Animals then were euthanized and hypothalami were collected. Coronal sections (16 µm) were cut through the A14 and A15 nuclei and a series of every sixteenth section was assessed for TH and cFos colocalization using a dual-immunoperoxidase procedure. T infusion increased circulating T ($p<0.01$), decreased mean LH ($p<0.01$) and increased LH interpulse intervals ($p<0.01$). T infusion increased the percent of TH cells expressing cFos in the A14 [$p<0.01$; 40.38% (84/208 TH cells) *vs* 21.13% (37/173 TH cells)] and in the A15 [$p<0.01$; 33.07% (209/632 TH cells) *vs* 7.92% (75/947 TH cells)]. These results show TH-containing neurons of the A14 and A15 are activated by T infusion and suggest they may be involved in steroid-induced inhibition of LH release in the male. (Supported by AG-37203-8177).

745.3

NONGENOMIC TEMPORAL ACTION OF THE NEUROACTIVE STEROID, 3α-HYDROXY-4-PREGNEN-20-ONE (3αHP) IN GnRH-INDUCED SUPPRESSION OF FSH IN PERIFUSED RAT PITUITARY CELLS. J. P. Wiebe* and M. Wolfe. Hormonal Regulatory Mechanisms, B&G Building, Univ. of Western Ontario, London, Ontario, Canada N6A 5B7.

The recently discovered neuroactive steroid, 3αHP, has been shown to selectively suppress GnRH-induced pituitary FSH release by actions at the level of the gonadotrope membrane/Ca^{2+} channel and the cell signaling pathway involving protein kinase C and Ca^{2+} mobilization (*Endocrinology* 125:41, 1989; 134:371, 1994; 134:377, 1994). To determine the time course of action of 3αHP in FSH suppression, pituitary cells from random cycling female Sprague-Dawley rats were allowed to attach to cytodex beads (4-5 days), transferred to perifusion columns, equilibrated for 1 h and then 10-min samples collected for 330 min. Cells received a 5 min pulse of GnRH (10^{-7}M) at 30 min (Cr; control) and at 270 min (Tr; treatment; Tr-0 = start of pulse). Various regimens of 3αHP and other steroids were applied in conjunction with the Tr pulse. FSH was determined in all samples by specific RIA. GnRH pulses resulted (about 20 min after start of pulse) in a short (10-15 min) 4-30-fold peak (above baseline) of FSH release. When a 5-min pulse of 3αHP (10^{-9}M) was applied at Tr-0, the GnRH-induced FSH release was completely suppressed. A 3αHP pulse at Tr-0.5, Tr-5, Tr-7, Tr-10, and Tr-13, the GnRH-induced FSH release was 0-10%, 5-20%, 50-70%, 80-100%, and 90-100% of the Cr peak, respectively. A 5-min pulse of 3ßHP, progesterone, or estradiol at Tr-0 resulted in 100, 155, and 245%, respectively of the Cr FSH peak. Pretreatment of cells with estradiol prevented the suppressive action of 3αHP. The results indicate that 3αHP most effectively suppresses GnRH-induced FSH release when present at the start of GnRH release and in the absence of estradiol. They suggest that 3αHP action may be at the level of the GnRH-receptor binding. (Supported by NSERC of Canada)

745.4

CLONING AND SEQUENCING OF cDNA FOR HAMSTER GnRH. H.T. Jansen*, S.J. Berriman, P.J. Stevens, P. Zeitler, and M.N. Lehman. Dept. Cell Biology, Neurobiology, and Anatomy, Univ. Cincinnati, Coll. Med. and Children's Hospital Research Foundation, Cincinnati OH 45267.

Reproductive activity in a number of species undergoes marked seasonal reversals as a result of changes in the activity of neurons secreting the decapeptide GnRH. In the golden hamster, a seasonal breeder, photoperiod-induced changes in GnRH gene expression have been described (Ronchi et al., *Neuroendocrinol.* 55:146, 1992). The identity of elements within the GnRH gene responsive to photoperiodic influences is unknown, in part, because the GnRH gene of this species has not been cloned. We have used the technique of RT/PCR to amplify, clone and sequence a partial cDNA of GnRH precursor in the golden hamster. Reverse transcribed total RNA from individual adult male hypothalami was amplified using the following oligonucleotide primers: 1) forward, an 18*mer* homologous to the highly conserved GnRH coding sequence within exon 2 in human, mouse, and rat and 2) reverse, a 17*mer* homologous to the conserved 5' end of exon 4 encoding the carboxy terminus of GnRH-associated peptide (GAP) in the same 3 species. The expected 192 bp product containing the GnRH and GAP coding sequences was amplified, cloned, and subsequently sequenced. The results indicate that the nucleotide sequence of the hamster GnRH coding region shares 98%, 93%, and 95% homology with human, rat, and mouse, respectively; the predicted peptide sequence shares 100% homology with all three species. The predicted GAP peptide sequence (amino acids 1-50) shares 82%, 80%, and 82% homology with human, rat, and mouse, respectively. In situ hybridization histochemistry using an antisense oligonucleotide probe to GnRH revealed a distribution of labeled cells similar to that observed with immunocytochemical methods. Upstream regulatory sequences in the GnRH gene are being isolated and analyzed using hamster-specific probes. (Supported by USDA 9102515).

745.5

DEVELOPMENT OF TWO GNRH SYSTEMS IN NEURONAL AND IMMUNE CELLS OF A MAMMAL. C.J. Gill*, J.A. King, R.P.Millar and E.F. Rissman. Dept. of Biology, University of Virginia, Charlottesville, VA 22903

The development of neurons containing the mammalian molecular form of gonadotropin-releasing hormone (mGnRH) has been well described for several species, but the development of neurons expressing other GnRH molecular forms is less well understood. Neurons containing mGnRH in the musk shrew, *Suncus murinus*, are observed by immunocytochemistry (ICC) in the olfactory epithelium by embryonic day 15 (of a 30 day gestation) and appear to migrate in to their adult olfactory and forebrain positions before birth. The musk shrew is the only mammal known to have an additional population of GnRH neurons in the midbrain. These cells contain chicken II GnRH (cGnRH-II), the GnRH molecular form considered most ancient and most widespread among non-mammalian vertebrates. Neurons immunoreactive for cGnRH-II are not observed in the musk shrew brain until between postnatal day 2 and 5. At this time, they have assumed their adult positions in the midbrain. However, cGnRH-II containing mast cells are present throughout the brain, but primarily in the habenula, in embryos and postnatal animals.

The concentrations of both GnRH forms in embryonic and postnatal brain tissue were determined by radioimmunoassay (RIA). RIA revealed that mGnRH levels rise on the day before birth, drop on postnatal day 1 (P1), and increase again by P5. The amount of cGnRH-II is low but detectable in embryos and increases between the day of birth and P5. Though neuronal production below ICC detectability may explain these data, it is also likely that these levels of cGnRH-II detectable by RIA are produced by embryonic mast cells. These data suggest a previously unreported association between the development of the mammalian neuroendocrine and immune systems. This work was supported by NIH NRSA 1F31MH10570-01A1 and NSF grant IBN 94-12605.

745.6

EFFECTS OF AROMATASE INHIBITOR ON GNRH GENE EXPRESSION IN SHEEP C.S.J. Yang*, A. Benoit[1], M.S. Smith[2] and R. A. Dailey Div. Anim. & Vet. Sci, West Virginia Univ., WV 26505, [1]Depart. Anim. North Carolina State Univ. NC 27695 and [2]Depart. Neurobiol., Univ. Pittsburgh, Pittsburgh, PA 15261.

We reported a change in GnRH mRNA levels during the ovine estrous cycle. Specifically, levels were highest immediately preceding and lowest after the LH surge. To investigate the importance of the preovulatory peak of estradiol-17β (E_2) on postovulatory GnRH gene expression, we treated ewes with an aromatase inhibitor, CGS16949A (CGS, 1 mg/kg wt., i.v.) [Ciba Geigy, Ltd., Basal, Switzerland], beginning 30 min. prior to induction of luteolysis by $PGF_{2\alpha}$ on day 6 of the estrous cycle. The CGS treatment continued every 8 hr until injection of hCG (750 IU, i.m.) given 36 hr after induction of luteolysis. Ewes were sacrificed at either 4, 6, or 8 days (post surge) following hCG. Control animals received saline instead of CGS. Number of ewes sacrificed at each time were 4, 2, and 2 for CGS and 4, 4, and 4 for controls. Mean levels of E_2 in serum at 24 hr after $PGF_{2\alpha}$ averaged 2.66±1.28 for CGS and 7.44±2.51 pg/ml for controls. E_2 levels did not differ between groups when animals were sacrificed. *In situ* hybridization using an ^{35}S-labeled probe for human GnRH mRNA was used to determine the GnRH mRNA levels. Eight anatomically matched, serial sections (S, 40 µm) from the POA of each animal were analyzed by image analysis. Neither number of neurons expressing GnRH-mRNA nor average grains per cell differed with treatment (T), day (D) or T x D. The results indicated that treatment of CGS did not change the GnRH expression pattern. Overall groups, analysis of GnRH neuronal numbers showed an interaction between D and S ($p<0.02$), and levels of E_2 were correlated negatively ($r= -0.35$, $p =0.12$) with number of neurons. Preovulatory levels of E_2 apparently do not play a key role in regulating postovulatory gene expression for GnRH in sheep. Supported by Hatch 321 & HD14643

745.7

A DIRECT PROJECTION FROM THE LATERAL SEPTUM TO GnRH NEURONS IN THE RAT. F. Varoqueaux[a], P. Ciofi[a], G. Tramu[b], J.C. Beauvillain*[a] and P. Poulain[a]. [a]U422 INSERM, Place de Verdun, 59045 Lille (France); [b]Lab. Neurocyto. Fonct., URA CNRS 339, Univ. Bordeaux I, 33405 Talence (France).

The lateral septum (LS) relays hippocampal information to a variety of hypothalamic areas modulating the neuroendocrine output. Especially, the LS has long been known to be involved in the regulation of reproductive processes. In line with this and using a tract-tracing technique, we investigated the existence of a direct projection from the LS to neurons of the gonadoliberin (GnRH)ergic apparatus.

Nine adult male rats (400g, b.w.) received a unilateral microiontophoretic injection of a biotinylated-dextran into the ventral LS and anterograde transport was allowed to proceed for 15 days. Brains were fixed (4% paraformaldehyde) and serially cut on a freezing microtome. All sections were doubly-stained to reveal the LS projection fibers with the avidin/peroxidase-diaminobenzidine technique and the GnRH neurons using immunofluorescence. Appositions between LS efferents and GnRH-immunoreactive (ir) neuronal profiles were counted at the light microscopic level.

In all cases such appositions suggestive of potential contacts were found to involve 20-30% of the GnRH-ir neurons located ipsilateral to the injection site. There was no obvious dorso-ventral or antero-posterior topographic arrangement of the contacted neurons that were found in the preoptic region as well as in the suprachiasmatic hypothalamic continuum. Potential contacts between labeled LS efferents and GnRH-ir neurons were observed on the proximal dendrites as well as the perikaryon of the GnRH-ir neurons. The majority (80%) of the contacted GnRH-ir neurons were of the irregular sub-type. The synaptic nature of these appositions between LS efferents and GnRH neurons is presently under study at the ultrastructural level.

These results suggest the existence of a direct, monosynaptic projection from the LS to a sizeable component of the GnRHergic neuroendocrine apparatus. The nature of the LS information relayed to the GnRH apparatus remains to be clarified.

Supported by INSERM.

745.8

TIME COURSE STUDY OF GNRH AXONAL OUTGROWTH FROM THE PREOPTIC AREA (POA) TO THE MEDIOBASAL HYPOTHALAMUS (MBH) IN ORGANOTYPIC CULTURES. M-C. Rogers*, A-J. Silverman[1], M.J. Gibson. Dept of Medicine and Fishberg Center for Neurobiology, Mount Sinai School of Medicine, NY, NY10029. [1]Dept of Cell Biology and Anatomy, Columbia University, NY, NY10032.

GnRH terminals project *in vivo* to the median eminence (ME) of the MBH along a stereotypic route. When hypogonadal mice receive an embryonic POA graft in the third ventricle, GnRH outgrowth to the ME may be seen as early as 5 days after implantation. In our organotypic culture system tissue was placed inside an insert chamber on a porous membrane coated with collagen and laminin, and fed by capillarity from the underlying defined media. When E15 POA was cultured with MBH and processed for GnRH immunoreactivity 7 days later, significant GnRH axonal outgrowth was observed on the side of the POA directly facing the MBH co-explant as compared with the side away from the MBH ($p<0.0001$). Such differential outgrowth was absent with control co-explants (Endo. Soc. Abst. 1995). This suggests the presence of an MBH-derived chemotropic diffusable substance attractive for GnRH axons. In the present study, when explants were cultured from 1 to 10 days, we documented increased GnRH axonal outgrowth from the POA over time. The number of GnRH fibers was significantly higher on the side facing the MBH after 4, 7 and 10 days of culture ($p<0.01$). At 1 day of culture the number of GnRH fibers was too low to detect significant preferential outgrowth. In addition to being more numerous after 10 days of culture, GnRH fibers appeared longer, often entering the MBH explant. GnRH cell number decreased by 13.9% between 1 and 4 days of culture, by 18.2% between 4 and 7 days, and remained stable thereafter. Preferential GnRH outgrowth to the MBH was observed throughout the time course indicating that the MBH chemotropic action was maintained as long as 10 days in culture. We have observed unidentified cells and processes on the membrane between the co-explants. Further experiments are under way to distinguish potential chemotropism from the possible role of such cellular elements.

Supported by NIH grant: NS 20335

745.9

POST-TRANSCRIPTIONAL REGULATION OF THE GONADOTROPIN-RELEASING HORMONE (GnRH) GENE IN GT1-7 CELLS: mRNA TURNOVER. T.J. Wu*, A.C. Gore and J.L. Roberts. Fishberg Center for Neurobiology, Mt. Sinai School of Medicine, NYC, NY 10029.

Several groups have reported that treatment of GT1-7 cells with phorbol myristate acetate (PMA), a phorbol ester, caused a decrease in the cytoplasmic GnRH mRNA concentration. While there is an inhibitory transcriptional effect of PMA, there also appears to be an effect on enhancing GnRH mRNA degradation. In support of this latter mechanism, we also found that PMA treatment resulted in a time-dependent decrease in the length of the GnRH mRNA poly-A tail ($t_{1/2} \approx$ 8 h), an index of mRNA turnover. We hypothesized that there must exist regions of the GnRH mRNA that serve as recognition signal targets for the degradation of the mRNA. The mechanism(s) mediating this alteration of mRNA stability is unknown, but previous work in other systems indicates that labile protein(s) are involved in the alteration. To this end, we have identified three potential regions of the GnRH mRNA, two hairpin loops (+128 to +156 and +302 to +328) in the translated region and an AU-rich sequence in the 3'-untranslated region (3'-UTR) (+395 to +422), that may serve in the regulated destabilization of the mRNA. We report the identification of cytosolic factors that bind to the GnRH mRNA using gel mobility shift assays and competition experiments. Serum treatment of cells, which inhibits GnRH mRNA levels, gave cytosolic extracts with attentuated protein binding to the second hairpin loop and the 3'-UTR. These findings should aid in our understanding of how the cell selectively targets the GnRH mRNA for turnover. [DK-08743 (ACG), DK-39029 (JLR)]

745.10

GnRH mRNA IS RAPIDLY TURNED OVER *IN VIVO*. J.L. Roberts*, S. Wray#, and A.C. Gore. Fishberg Res. Center for Neurobiology, Mt. Sinai Medical Center, New York, NY 10029-6574, and #Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

Previous studies analyzing GnRH gene expression *in vivo* and *in vitro* have suggested that there are rapid, post-transcriptional mechanisms involved in its regulation. Interestingly, in the GT1 cells there is a very slow turnover of GnRH mRNA with a half-life of 1-2 days. However, rapid changes in GnRH gene expression in the animal are observed following neurotransmitter or steroid manipulation, and an analysis of RNA turnover by *in situ* hybridization has suggested that turnover of GnRH mRNA occurs more rapidly in explant cultures than in GT1 cells. We have directly addressed this question in male rats using RNA synthesis inhibitors coupled with RNase protection assays to quantitate GnRH primary transcript and mRNA. Actinomycin D injected in the third ventricle or directly into the preoptic area (POA) had no effect on either nuclear or cytoplasmic GnRH RNA species. However, injection into the POA of the water-soluble RNA polymerase II-specific inhibitor dichloro-ribofuranosylbenzimidazole (DRB) caused a significant inhibition in GnRH nuclear RNA primary transcript levels and a subsequent inhibition of cytoplasmic GnRH mRNA. These studies have suggested an upper limit of 6-8 hr for the half-life of the cytoplasmic mRNA. We are currently pursuing similar studies in cultured postnatal hypothalamic explants in which a greater percentage of the GnRH neurons are exposed to DRB. These studies suggest that GnRH mRNA turnover may be a crucial mode of regulation of GnRH gene expression in the intact animal, and differs from that observed in GT1 cells.

745.11

LHRH GENE EXPRESSION IN POSTNATAL CNS SLICE EXPLANT CULTURES: EXAMINATION OF LHRH mRNA TURNOVER. J. A. Maurer and S. Wray*. Laboratory of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

Using the immortalized LHRH cell line, GT1-7, investigators have reported negative regulation of LHRH gene expression by phorbol 12-myristate 13-acetate (PMA) treatment. Our laboratory saw a similar regulation of LHRH gene expression in postnatal hypothalamic slice explant cultures. In primary LHRH neurons, LHRH mRNA dramatically decreased after a 4 h exposure to either forskolin or PMA. We hypothesized that these changes were the result of increased degradation of LHRH mRNA. To investigate LHRH mRNA stability, actinomycin D, a non-specific blocker of RNA polymerases, and 5,6-dichloro-1-D-ribofuranosyl-benzimidazole (DRB), a selective inhibitor of RNA polymerase-II, were used to inhibit transcription in postnatal LHRH neurons maintained in long-term organotypic roller cultures. Two days prior to treatment, cultures were incubated with 1 µM tetrodotoxin (TTX) to suppress spontaneous electrical activity. On day 18 of culture, in addition to TTX, explants received 4 µM actinomycin D or 150 µM DRB for 2 to 16 h and then processed for *in situ* hybridization histochemistry or immunocytochemistry. Under all treatment conditions numerous LHRH immunopositive neurons were detected suggesting that levels of LHRH peptide are not dramatically altered by treatment with transcription inhibitors. Preliminary data from single cell analysis indicate that exposure to DRB for 2 to 4 h decreases LHRH mRNA. These results are consistent with the hypothesis that second messengers regulate LHRH gene expression by increasing LHRH mRNA turnover.

745.12

LONG-TERM EFFECTS OF OVARIECTOMIES IN FEMALE RATS ON RADIAL ARM MAZE PERFORMANCE USING LONGITUDINAL AND CROSS-SECTIONAL DESIGNS. A.C. Bartolomeo, J.A. Moyer, E.A. Muth* and C.A. Boast. CNS Disorders, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

Recent clinical studies have suggested a connection between reduced levels of estrogen and cognitive deficits, especially in Alzheimer's Disease. To determine if estrogen depletion would lead to memory impairments, a longitudinal design was initiated with ovariectomized (OVX) and intact (INT) female Sprague-Dawley rats, in a one hour delay, non-match-to-sample Radial Arm Maze (RAM) task. Subjects had pre-surgical RAM training. Post-surgical (PS) testing started at one week and subjects were retested at four month intervals through 24 months PS. There were no differences in mean post-delay errors (PDE) between groups at any of the times although PDE increased with age. Scopolamine (0.03 - 0.54 mg/kg, s.c.), a cholinergic antagonist, was administered to half of the OVX and all of the INT rats following testing at each time point. At 3 weeks PS and 4 months PS, 0.54 mg/kg SCOP produced significantly more PDE in both treated groups than in OVX-SAL, but there were not any differences in PDE between the treated groups at any of the time points. At 8 months PS, the OVX-SCOP group had more animals unable to complete the pre-delay than the INT-SCOP. At 12 months PS and beyond, there was no difference in the number of rats unable to complete the pre-delay in both treated groups. MK-801 (0.03 - 0.1 mg/kg, i.p.), a non-competitive NMDA antagonist, was administered subsequent to SCOP testing beginning at 16 months PS. At 16 months PS, 0.03 mg/kg MK produced significantly more PDE in both treated groups than in OVX-SAL, but there were not any differences in PDE between the treated groups at any time point. In the second study, a cross-sectional design, naive rats were tested at 4, 8 or 11 months PS in the RAM task. PDE were not significantly different between the OVX and INT at the earlier points, but at 11 months, the INT group consistently made more PDE with statistical significance attained on 5 of 13 trials. Long-term depletion of ovarian hormones does not appear to induce cognitive deficits in rats in a spatial working memory task.

745.13

CELLULAR ANALYSIS OF TYROSINE HYDROXYLASE EXPRESSION IN LACTATING RATS K.A. Berghorn*, B.J. Duska, H.-J. Wang, W.-W. Le, T.G. Sherman and G.E. Hoffman. Departments of Neurobiology and Neuroscience, University of Pittsburgh, Pittsburgh, PA, 15261.

Earlier studies conducted in our laboratory have detected marked reduction in tyrosine hydroxylase (TH) gene expression in the dopamine neurons of the arcuate nucleus during lactation with a resurgence of expression following pup removal. Analysis of the level of TH gene expression by *in situ* hybridization (ISH) using ^{35}S labeled riboprobes did not permit analysis of changes of mRNA levels within individual cells owing to the density of cell clusters and spread of the radioactive signal beyond cell borders. Application of sensitive non-radioactive methods for ISH with biotin labeled riboprobes has enabled detection of granular mRNA label within the cell cytoplasm that appears to reflect accurately the ribosomal compartment without spread into other cellular compartments. Using this approach, we examined the pattern of change in TH mRNA levels in cycling rats and in lactating rats nursing 8 pups and following pup removal. Changes in TH mRNA levels detected with the biotin labeled probes generally paralleled those observed with ^{35}S labeled riboprobes: mRNA levels in the lactating animals nursing their pups were at or below the limit of detection in most arcuate TH neurons, and levels of TH mRNA in the arcuate nucleus increased beyond diestrous levels when pups had been removed for 24-48 hrs. Moreover, by use of double labeling of the TH mRNA and TH protein, we could accurately locate the transcriptionally silent TH population in each condition and define which subpopulations of arcuate dopamine neurons were stimulated transcriptionally after removal of the suckling stimulus. *(Supported by NIH NS 28730 and NSF BNS 9021307).*

MOTOR CORTEX: FUNCTIONAL ORGANIZATION AND PLASTICITY I

746.1

INTERHEMISPHERIC ASYMMETRIES IN 2-DEOXYGLUCOSE UPTAKE AND OPEN-FIELD BEHAVIOR OF CALLOSAL AND ACALLOSAL MICE. F. Magara, E. Welker*†, D.P. Wolfer and H.-P. Lipp. Inst. of Anatomy, Univ. of Zürich, †Inst. of Anatomy, Univ. of Lausanne, Switzerland.

In order to study functional lateralization in mice, we measured the uptake of 2-deoxyglucose (2DGU) in the left and right side of the brain of mice while they were freely moving in an open-field. Ten C57BL/6 mice and 12 sex- and age-matched mice with callosal agenesis were injected i.p. with a solution of 1-[14C]-2-Deoxy-D-Glucose (2DG), and immmediately released in a dimly illuminated open-field arena (ø = 150 cm). Their paths were recorded and analysed off-line to assess activity, turning preferences and distance to the wall as a measure for exploration versus anxiety. After 45 min., the mice were sacrificed, and the brains processed for contact autoradiography. The amount of 2DGU in anatomically defined regions (30 subcortical and 11 cortical) was assessed by means of a computer-aided image analysis. Acallosal mice showed a high propensity for circling, the degree of turning preferences to the left or to the right being significantly correlated with 2DGU-asymmetries in the substantia nigra and the diagonal band of Broca. These correlations were missing in the callosal C57 mice. In both groups of mice, the 2DGU asymmetry in the lateral amygdala was correlated with thigmotaxis, animals with high activity in the right amygdala spending most time in the center of the open-field. We conclude that the presence of a corpus callosum helps to correct asymmetrical influences from ascending activation systems on locomotor directedness. Independent of the corpus callosum, there appears to be functional lateralization of fear-related processes in the amygdala. Supported by Swiss National Science Foundation 31-37497.

746.2

EYE, HEAD, BODY AND FORELIMB MOVEMENTS EVOKED FROM THE ANTERIOR ECTOSYLVIAN CORTEX OF THE UNRESTRAINED CAT. H. Jiang* and D. Guitton. Montreal Neurological Institute and McGill University, Montreal, Quebec, Canada, H3A 2B4.

The anterior ectosylvian cortex of the cat receives different sensory inputs and has been considered an area for sensor-motor integration. We recorded sensory responses in neurons along the banks of the anterior ectosylvian sulcus of an alert cat and investigated possible motor correlates by electrically micro-stimulating (~60 μA) recording sites. In the head-fixed condition, stimuluation evoked eye movements that were driven to a fixed contralateral orbital position. When the head was freed, with body fixed, the same stimulus drove gaze (eye+head) shifts towards a fixed contra-position relative to the body: both the amplitude and direction of evoked gaze movements were modulated by initial gaze position. When the body was freed, the same stimulus drove coordinated rapid gaze and contralateral reaching forelimb (paw) movements, and body postural adjustments. Gaze looked at, and paw went to, a fixed contra-spatial location relative to the initial body axis. For movements beginnng with gaze, head and body aligned, the location of visual receptive fields and the preferred direction of moving visual targets were correlated with the final position of evoked gaze shifts and paw movements. The cortical area which evoked eye, head and forelimb movements was located mainly in the middle one-third of the ventral bank and the fundus slightly extending to the dorsal bank. A topographic representation of 3-D space re-body may exist in this cortical area. We do not know whether stimulation generated a 'target' that the animal tried to "catch" or an eye-head-limb motor program.

746.3

FUNCTION OF MASTICATION-RELATED NEURONS WITH RHYTHMICAL BURST FIRING IN THE OROFACIAL FIRST SOMATOSENSORY CORTEX OF CONSCIOUS CATS. HIRABA, H.*, YAMAGUCHI*,Y., KAMOGAWA, H. and SUMINO, R. Dept. of Physiol., Div. of Pathophysiol.*† Nihon Univ. Sch. of Dent., Tokyo 101, Japan

We have studied the activity patterns of mastication-related neurons (MRN) in the orofacial first somatosensory cortex (SI) of conscious cats. Over 90% of MRNs in the orofacial SI showed activity changes that followed the masticatory rhythm (RB-MRN). About 20% and 40% of RB-MRNs received inputs from the tongue and perioral regions. The RB-MRNs with the receptive fields in the tongue (T-PB-MRN) and perioral regions (P-RB-MRN) showed the regular burst firing corresponded to the jaw-opening to -opened and the jaw-closed phases during food intake respectively. But they showed the irregular one after food intake. T-RB-MRN showed also firing frequency changes in burst firing that were dependent on differences in food texture, but P-RB-MRN did not. Furthermore, to investigate the function of T- and P-RB-MRNs during mastication, we examined the effects of each lesion of perioral or tongue projection area in SI on masticatory behavior.Lesions were created by injection of kainic acid (1%, 2μl). After creation of each lesion, we observed the resulting deficit of orofacial behavior during mastication for 5-6 weeks. We then compared the difference in behavior caused by the lesions in the perioral and tongue projection areas (P-L and T-L animals). P-L animals showed a delay in the start of mastication and the dropping of ingested food during mastication. T-L animals showed the prolongation of the masticatory process. On the basis of these findings, we will discuss the function of sensory information in the orofacial SI for the performance of mastication.

746.4

SENSORIMOTOR CORTEX RESPONSES TO FACIAL NERVE STIMULATION IN RATS. J. Okada, S. S. Suzuki*, Y. Harada1 and N. Nagamura. Biosignaling Dept., Natnl. Inst. Biosci. and Human-Technol., Tsukuba 305, and 1Dept. Physiol., Nippon Med. Sch., Tokyo 113, Japan.

The present study was designed to examine the basic characteristics of facial nerve-evoked neural activities in the primary somatosensory (SI) and motor cortex (MI). Under anesthesia, adult rats were implanted with a stimulating electrode on the buccal branch of the left facial nerve. Field potentials (FP) and multiple unit activity (MUA) evoked by facial nerve stimulation were recorded. Facial nerve stimuli elicited primarily negative or negative-positive potentials in layers II-V bilaterally in SI and MI. Large negativities were always accompanied by a salient MUA indicating a synchronous excitation of local neurons. The primary response latency was 4-9 ms for contralateral SI, 9-10 ms for contralateral MI and 13-15 ms for ipsilateral SI/MI. Current source density (CSD) analyses of laminar FP profiles in contralateral SI revealed two primary sinks at 400-800 μm (E1a) and 1000-1200 μm (E1b) from the cortical surface. E1a and E1b were followed by a secondary sink in more superficial (200-600 μm, E2a) and deeper layers (1200-1400 μm, E2b), respectively. Primary sinks in MI were distributed widely at 200-1200 μm. The relationship between the facial nerve-evoked FP map of MI and the output map of MI as revealed by intracortical microstimulation (ICMS) was examined in the same rats. Facial nerve-evoked potentials were obtained not only from the ICMS-defined vibrissal region of MI but also from adjacent regions including the forelimb, jaw and tongue regions. Furthermore, medial parts of the vibrissal region showed no or small responses to facial nerve stimulation.

746.5

CORTICAL ACTIVATION OF ORAL MOVEMENTS IN RATS. R.E. See* and A.M. Lynch. Department of Psychology, Washington State University, Pullman, WA, 99164-4820.

Previous studies using electrical stimulation have demonstrated the role of the primary oral motor cortex in regulating oral behaviors. The present experiment examined changes in oral movements after direct pharmacological stimulation of the primary oral motor cortex in the awake, unrestrained rat. Male, Sprague-Dawley rats were implanted with bilateral infusion cannulae (22 gauge) aimed at the primary oral motor cortex (A +3.5, L ±3.5, V -1.5). Following one week recovery, animals were tested in observation chambers for motor activity. Infusion of N-methyl-D-aspartate (NMDA) and the $GABA_A$ receptor antagonist, picrotoxin, produced a concentration dependent increase in orofacial activity. Motor activity consisted primarily of nondirected chewing movements and tremor of the masseter muscles. In addition, both teeth grinding and directed biting were also observed. The effect was selective to oral behavior in that other motor activity did not show a significant increase. These findings demonstrate that a discrete orofacial motor syndrome can be directly elicited by pharmacological probes into the oral motor cortex. The relationship of these movements to subcortical oral motor pathways will be discussed (Supported by NIH DE09678).

746.6

EFFECTS ON MASTICATION OF REVERSIBLE COLD BLOCK OF LATERAL PERICENTRAL CORTEX OF AWAKE MONKEY. N. Narita, B.J. Sessle, R. Raouf and C.-S. Huang* Faculty of Dentistry, University of Toronto, Toronto, M5G 1G6, Canada

Our laboratory has shown that intracortical microstimulation (ICMS) of a region of the lateral pericentral cortex can evoke masticatory-like movements in awake monkeys (Huang et al., J. Neurophysiol. 61; 635, 1989). The aim of this study was to determine if mastication could be affected by reversible cold block-induced inactivation of this ICMS-defined region. Two cranial chambers were chronically implanted in the monkey (M. fascicularis). A warm or cold alcohol-water solution was pumped through thermodes placed bilaterally on the dura overlying the lateral pericentral cortex while the monkey chewed standardized amounts of fruit during pre-cool (thermode temperature 37°C), cool (0°C), and rewarm (37°C) conditions. Electromyographic (EMG) activity was recorded from masseter (MA), anterior digastric (AD) and other orofacial muscles, and video recordings made of orofacial movements. The chewing cycle and preparatory phase of rhythmic chewing were significantly prolonged during cold block (mean ± SD, 0.51 ± 0.12s and 2.26 ± 0.93s, respectively, ANOVA, $p<0.05$) compared to pre-cool (0.31 ± 0.06s and 0.75 ± 0.20s) and rewarm (0.32 ± 0.04s and 0.90 ± 0.19s) conditions. EMG burst durations were also significantly prolonged during cold block (MA: 0.35 ± 0.12s, AD: 0.33 ± 0.11s, ANOVA, $p<0.05$) compared to pre-cool (MA: 0.23 ± 0.04s, AD: 0.19 ± 0.03s) and rewarm (MA: 0.25 ± 0.03s, AD: 0.20 ± 0.03s) conditions, and EMG amplitudes were also significantly reduced (MA: 0.82 ± 0.21, AD: 1.74 ± 0.48, A/D units, ANOVA, $p<0.05$) compared to pre-cool (MA: 1.06 ± 0.24, AD: 2.19 ± 0.42, A/D units) and rewarm (MA: 0.98 ± 0.23, AD: 2.20 ± 0.43, A/D units) conditions. These data provide further evidence that the lateral pericentral cortex plays a critical role in the initiation and regulation of primate mastication. Supported by Canadian MRC grant MT-4918.

746.7

EFFECTS OF REVERSIBLE COLD BLOCK OF LATERAL PERICENTRAL CORTEX ON SWALLOWING IN AWAKE MONKEY. B.J. Sessle, N. Narita and R.E. Martin*† Fac. of Dentistry, Univ. of Toronto, Toronto, M5G 1G6 and Fac. of Appl. Health Sciences, Univ. of Western Ontario, London, N6G 1H1†, Canada.

We have recently shown that swallowing can be evoked in the awake monkey by intracortical microstimulation (ICMS) of a region of the lateral pericentral cortex (Martin et al., Neurosci. Abstr. 19; 777, 1993). This study was initiated to determine the effects on swallowing of reversible cold block-induced inactivation of this ICMS-defined region. Two cranial chambers were chronically implanted in the monkey (M. fascicularis). A warm or cold alcohol-water solution was pumped through thermodes placed bilaterally on the dura overlying the lateral pericentral cortex while the monkey swallowed following mastication of standardized amounts of fruit, or following sucking or licking of standardized amounts of juice, during pre-cool (thermode temperature 37°C), cool (0°C), and rewarm (37°C) conditions. Electromyographic (EMG) activity was recorded from masseter (MA), genioglossus (GG), anterior digastric (AD), geniohyoid (GH) and thyrohyoid (TH) muscles, and video recordings made of orofacial movements. The incidence of swallowing following chewing was significantly decreased during cold block (14.8%, χ^2-test, $p<0.0001$) compared to pre-cool (88.5%) and rewarm (100%) conditions. Swallow-related EMG activity following mastication, sucking and licking was also affected, e.g. EMG burst duration and amplitude of TH following licking were significantly (ANOVA, $p<0.05$) modulated during cold block (mean ± SD, 0.75 ± 0.35s and 4.75 ± 1.11 A/D units, respectively) compared to pre-cool (0.40 ± 0.04s and 7.62 ± 0.68 A/D units) and rewarm (0.44 ± 0.05s and 7.06 ± 0.91 A/D units) conditions, and the interval between MA and TH swallow-related EMG onset following mastication was significantly increased (0.18 ± 0.06s, ANOVA, $p<0.05$) compared to pre-cool (0.11 ± 0.03s) and rewarm (0.13 ± 0.02s) conditions. These data provide further evidence that the lateral pericentral cortex plays a critical role in the initiation and regulation of swallowing in the primate. Supported by Canadian MRC grant MT-4918.

746.8

MODULATION OF OPTICAL INTRINSIC SIGNALS BY INTRODUCTION OF A COMPETING STIMULUS

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Optical intrinsic signal imaging was used to detect reflectance changes in two regions of rat somatosensory cortex. Possible interactions of activity between barrel cortex and the forelimb region of the primary somatosensory cortex were investigated. This was accomplished by evaluating intrinsic signal response magnitude and spatial extent in response to simultaneous peripheral stimulation of single whiskers and forelimb digits.

Whisker C1 was deflected at a frequency of 10 Hz for 1 second while vibratory stimuli of varying intensities were applied to forelimb digits. Intrinsic signal responses to simultaneous whisker and forelimb stimulation were compared to whisker stimulated controls. Addition of the forelimb stimulus modulated intrinsic signal activity by increasing or decreasing the magnitude and/or spatial extent of responses over barrel cortex. The degree of modulation was related to the intensity of the competing stimulus. These data suggest that cortical responses at the level of primary sensory processing may be significantly modified by activity in adjacent regions.

746.9

PROCESSING OF IPSILATERAL SOMATOSENSORY AFFERENTS IN PRIMARY MOTOR CORTEX OF THE MACAQUE. M.C. Lee* and J.C. Arezzo. Departments of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

Direct excitatory afferents from the contralateral hand to primary motor cortex have been demonstrated in single unit studies in primates. The contribution of activity evoked by ipsilateral distal median nerve stimulation was explored to investigate potential excitatory or inhibitory interactions between the bilateral afferents in this area. Local populations of neurons in the hand representation of area 4 in two cerebral hemispheres of an awake adult macaque monkey were sampled with a low impedence (300-500kOhm), multicontact electrode (150-200μ spacing). Somatosensory evoked potentials (SEPs) and concomitant multiunit activity (MUA) were measured simultaneously from up to fifteen sites within and across laminae, and one-dimensional current source density patterns (CSD) were calculated. Stimulation of the contralateral hand elicits an intracortical SEP characterized by an inverting potential, P17/N17, which onsets at about 8ms. This component is associated with a coincident excitatory burst of MUA which returns to baseline at approximately 50ms and is followed by a subtle reduction in MUA. Stimulation of the ipsilateral hand, however, elicits a markedly different response, characterized by a smaller excitatory burst of MUA at about 12ms. This is followed by a prolonged and striking 20-30% reduction below the spontaneous firing level in MUA from about 50 to 110ms. The laminar distribution of MUA and associated current flow in the ipsilateral response is consistent with a pattern of excitatory input followed by the active hyperpolarization of apical dendrites of infragranular pyramidal cells. While the timing of activity suggests that the excitatory inputs are direct rather than callosal, the origins of the inhibitory activity have yet to be elucidated. These data support the hypothesis that primary motor cortex is active in response to somatosensory stimuli, and suggest that the ipsilateral afferents may serve to sharpen the boundary between afferents from contralateral and ipsilateral hands. Supported by MH 06723.

746.10

VESTIBULAR AND OPTOKINETIC SPATIAL TUNING IN NEURONES OF THE PARIETO-INSULAR VESTIBULAR CORTEX IN THE SQUIRREL MONKEY. W. Guldin* and O.-J. Grüsser. Dept. of Physiol., Freie Universität Berlin, 14195 Berlin, Germany

The response characteristics of vestibularly driven units in the parieto-insular vestibular cortex (PIVC) have been tested in four alert squirrel monkeys. Sinusoidal vestibular and optokinetic stimulation was applied in eight different planes orthogonal to the yaw plane. Various forms of somatosensory stimulation were applied, such as brushing the skin, pressing different muscles, moving different joints. The neck-muscle input was tested by moving the body sinusoidally while the head was fixed in space. In the PIVC-region of the squirrel monkey about 60 percent of the units were driven by vestibular stimuli. Frequently vestibular neurones also responded to neck-muscle stimulation, input from other muscles or tendon or joint receptor stimulation. One third of the PIVC-units responded to optokinetic or small-field visual movement stimulation. Polar histograms of spatial tuning of the vestibular driven units in the PIVC showed, as a rule, a sector of maximal response around a preference plane. These preferred response planes did not correspond in most cases to the planes of the semicircular canals. Our results give evidence that the network of the PIVC cells can be interpreted as a cortical integrator of vestibular input from all semicircular canals. The PIVC processes information from the vestibular, somatosensory and optokinetic systems, which is useful in calculating the movement of the head in space and in relation to the other parts of the body.

746.11

MOVEMENT REPRESENTATION IN PRECENTRAL MOTOR AND PREMOTOR CORTEX OF OLD WORLD MONKEYS. T.M. Preuss*, I. Stepniewska and J.H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

The lateral premotor cortex of Old World monkeys has not previously been examined in detail using intracortical microstimulation. We used this technique to map the distribution of motor responses across the precentral gyrus in 8 Old World monkeys (7 *Macaca* spp., 1 *Erythrocebus patas*). Animals were anesthetized with ketamine and xylazine and current delivered with tungsten microelectrodes.

We were able to elicit responses reliably from both the dorsal and ventral premotor areas (PMD, PMV), identified architectonically, as well as from caudal and rostral divisions of the primary motor area (M1c, M1r). PMD was less responsive to stimulation than PMV or M1, but even in PMD, many points were responsive with currents of 40—100 μA. We elicited hindlimb, axial, forelimb, and facial movements from both PMD and M1, but only forelimb and facial movements after stimulation of PMV. Thus, we found no evidence of hindlimb representation in PMV. The forelimb representation of PMV is bordered medially by a zone of facial and upper axial responsiveness located in lateral-most PMD. Stimulation yielded both proximal and distal forelimb movements from PMD, but predominantly distal movements from PMV. These results are largely consistent with our previous microstimulation studies in owl monkeys, indicating that stimulation of premotor cortex can yield discrete motor responses. Moreover, our results do not support the widely held view that there is a caudal-to-rostral gradient of distal-to-proximal-to-axial representation in precentral cortex.

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MOTOR CORTEX: FUNCTIONAL ORGANIZATION AND PLASTICITY II

747.1

QUANTITATIVE CHANGES OF GABA-IMMUNOREACTIVE CELLS IN THE HINDLIMB REPRESENTATION OF THE RAT SOMATOSENSORY CORTEX AFTER 14-DAY HINDLIMB UNLOADING BY TAIL SUSPENSION. F. D'Amelio,[1]* L. C. Wu,[1] R. A. Fox,[1] and N. G. Daunton,[2] Dept. Psych.,[1] San José St. Univ., San José, CA 95192-0120, and NASA-Ames Res Ctr.,[2] Moffett Field, CA 94035-1000

In the present study γ-aminobutyric acid (GABA) immunoreactivity was evaluated quantitatively in the hindlimb representation of the rat somatosensory cortex after 14 days of tail suspension (TS). The number of GABA-immunoreactive cells was reduced in cortical layers IV, Va, Vb and VI ($p<0.05$, $p<0.0002$, $p<0.02$, and $p<0.03$ respectively) of rats subjected to TS. In addition, the number of GABA-containing terminals, particularly those terminals surrounding the soma and apical dendrites of pyramidal cells in layer Vb, also were reduced throughout the same cortical layers. Since there was no reduction in the total neuronal density of the hindlimb representation as compared with the control animals, we concluded that the reduction was not due to cell death. Findings are discussed in the context of previous morphological and behavioral studies of the neuromuscular system of TS animals and we propose that alterations in the reflex organization of hindlimb muscle groups that are triggered by TS elicit disturbances in the afferent signaling and feedback from intramuscular receptors to the cerebral cortex. We suggest that local circuit GABAergic neurons modulate cortical output in response to this altered afferent feedback.

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747.2

ROLE OF SOMESTHETIC INFORMATION IN THE PLASTICITY OF THE CEREBELLO-CORTICAL PATHWAY. E.M. Meftah and L. Rispal-Padel*. C.R. Cerveau et Cognition UMR 9940 Fac. Méd. 31062 Toulouse. France.

Long-lasting effects of somesthetic messages on cerebello-thalamo-cortical (CTC) transmission and on the motor responses induced by interpositus nucleus stimulation, were studied under associative conditions. Two α-conditioning procedures, in association with behavioral and electrophysiological approaches, were carried out on awake chronically prepared cats which had previously undergone a red nucleus neurotoxic lesion. In the 1st procedure, a sub-threshold activation of the CTC circuits controlling forearm flexion movements (conditional stimulus CS), was associated with a cutaneous stimulation (unconditional stimulus UCS) of the dorsum of the distal forearm. Both CS and UCS originally produce elbow flexions. In the 2nd procedure, the same CS was paired with another UCS, applied to a more proximal cutaneous receptive field and producing a backwards forearm withdrawal movement, different from that induced by the CS.

Under the 1st procedure, a persistent enhancement of the amplitude of the forearm flexions induced the CS was observed along with an increase of the cerebello-cortical excitatory responses in the elbow area of the motor cortex. In contrast, the 2nd procedure resulted in a decrease and the disappearance of the forearm flexions in favor of the appearence of forearm extensions, the amplitude of which increased concomitantly with a depression of the cerebello-cortical excitatory wave. A secondary inhibitory wave also developed in the cortical elbow area. These changes were long lasting and lead to the following conclusions: The CTC circuits are liable to undergo functional modifications in adult cats. The motor and CTC changes may be of two types depending on the somesthetic messages paired with the CS. The time courses of the changes affecting the CTC transmission and the motor responses are closely related.

747.3

PLASTICITY IN THE CEREBELLAR EFFERENT PATHWAYS. ROLE OF TEMPORAL RELATIONSHIPS BETWEEN CONDITIONAL AND UNCONDITIONAL STIMULATIONS IN THE INDUCTION OF PLASTIC CHANGES. M. Pananceau and L. Rispal-Padel. C.R. Cerveau et Cognition UMR 9940 Fac. Méd. 31062 Toulouse. France. (Spon: ENA*)

The properties of cerebellar output pathways can be modified by sensori-motor experience during the lifetime of an animal. It would appear that this plasticity depends on the convergence of motor-related neural activity with sensory information concerning the environment. The present study was aimed at elucidating the importance of the temporal relationships between such sensory and motor events in the development of these experience related changes. In chronic cats, the cerebello-thalamo-cortical and cerebello-rubral motor circuits controlling the forelimb were subjected to an associative conditioning procedure in which the interpositus nucleus (origin of both pathways) was subjected to sub-threshold conditioned stimulation (CS) in association with an unconditioned stimulus (UCS) which induced a reflex flexion movement of the elbow joint.The movements produced together with the responses in central structures were analysed during the course of the conditioning procedure. Three different CS-UCS delays were tested. When the CS was presented 100 ms after the UCS (UCS-CS 100) there was no effect on either the movement or the central responses. When the CS was applied either at the same time as the UCS (CS-UCS) or 100 ms before (CS-UCS 100), there was an increase in both the amplitude and the probability of the motor responses. In the CS-UCS situation, the motor changes were only transient, and dependent on changes in excitability that affected the entire motor cortex. In contrast, in the CS-UCS 100 situation, the changes were topical and long-lasting and were associated with a reinforcement of thalamo-cortical and cerebello-rubral synapses. Such changes were specific to circuits involved in controlling the elbow joint and were strongly correlated with the changes in the movement. They were only seen when the CS predicted the arrival of the UCS.

747.4

RAPID CHANGES IN THE ORGANIZATION OF SENSORY AND MOTOR EVOKED FIELDS FOLLOWING PERIPHERAL ISCHEMIA STUDIED BY MAGNETOENCEPHALOGRAPHY. M.Hund, A.Rezai, E.Kronberg, J. Cappell, U.Ribary*and R.Llinás. Dept. of Physiol. & Neurosci., NY Univ. Med. Center, 550 First Ave, NY, NY 10016.

Magnetoencephalographic (MEG) somatosensory and motor fields of the left hand (mostly middle finger) were recorded in five healthy subjects before, during and after nerve conduction block of their forearms using a pneumatic cuff. In addition, in two subjects the MEG-recording was repeated after total lidocaine block of the left middle finger. During the first 15 minutes of ischemia a 50% decrease of amplitudes of the sensory fields paralleled the progressive sensory loss. Surprisingly, a reduction in the motor field occurred as well. Source locations of the primary motor field components shifted within an area of approximately 1 cm^2. This occurred in all subjects and had a characteristic direction for each individual. Further attenuation of field components was seen in the following 15 minutes of ischemic nerve block. Frequency spectrum analysis showed a suppression of motor mu rhythm as well as a reduction in the 4 Hz band during this period. The fields regained their baseline coordinates and amplitudes 15 minutes after cuff removal. Lidocaine had effects on amplitudes, frequencies and dipole locations selectively on the deafferented middle finger similar to those of ischemia-induced anesthesia, while the non-anesthetized neighboring finger showed normal results. These findings imply that the ischemic effects are not due to cuff-related peripheral anoxia. The decrease of amplitudes in the motor field components during ischemia may be explained by reduced sensory afferent background activity. In summary, temporary peripheral deafferentation, either due to ischemic nerve block or to lidocaine-related digital block, leads to a rapid change in the frequency structure of sensory and motor field components.

747.5

MOVEMENT-RELATED NEUROMAGNETIC ACTIVITY DURING TRANSIENT DEAFFERENTATION. R. Kristeva-Feige, S.Rossi, V. Pizzella, A. Sabato, B.Feige, F. Tecchio, J. Edrich and P.-M. Rossini. Inst. Biomed. Engin., Univ. Ulm, 89069 Ulm, Germany. (SPON: EUROPEAN BRAIN AND BEHAVIOUR SOCIETY).

The present study was aimed at investigating the effect of blocking cutaneous input from the moving part of the body on movement-evoked field one (MEFI).

Neuromagnetic fields from the left cerebral hemisphere of three healthy, right-handed subjects were investigated preceding and accompanying voluntary right index finger movements under two different experimental conditions: before (stage A) and during (stage B) transient deafferentation. The last was achieved by anesthetic block of median and radial nerves at the wrist. In this way, during stage B cutaneous and part of the proprioceptive inputs relative to a wide hand area including the entire index finger were suppressed. The voluntary movements were not impaired because the muscles participating in the task were not influenced by the anesthesia.

The magnetic signals of the brain sources corresponding to the main components of the movement-related fields (motor field, MF and movement-evoked field I, MEFI) were mapped and localized by means of a moving dipole model. In the three subjects investigated, the MF and MEFI dipole sources in stage B were stronger (30% on average) than before anesthesia. No significant changes in component latencies and spatial co-ordinates of the estimated dipole sources between stages A and B were observed.

The results are discussed in terms of three hypotheses not mutually excluding each other: „efference copy" hypothesis, spatial attention hypothesis and plasticity hypothesis.

747.6

DIFFERENTIAL PATTERNS OF INTRINSIC MOTOR CORTEX PROJECTIONS AS A SUBSTRATE FOR CORTICAL MOTOR MAP PLASTICITY. J.W. Tullai* and G.W. Huntley. Fishberg Reseach Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029.

Reorganization of representational sensory and motor maps in the cerebral cortex can be induced quickly under activity-dependent conditions. In rat motor cortex, transection of the facial nerve leads to a rapid, limited expansion of parts of the forelimb representation into the former whisker representation (Sanes et al., PNAS 85;1988). The connections which mediate such changes may involve intrinsic, horizontally-oriented pyramidal cell axon collaterals which can extend for long distances in motor cortex and cross map boundaries. To investigate whether intrinsic axons are anatomically positioned to mediate and constrain the extent of rapid changes in cortical motor maps, we combined electrical stimulation techniques and anterograde tract-tracing methods in adult rats to correlate the patterns of intrinsic projections with the occurrence and extent of the forelimb border shift induced after facial nerve transection. After nerve cut, small, iontophoretic injections of anterograde tracer (HRP or biocytin) were placed either into former whisker regions exhibiting novel forelimb activity, or into former whisker regions adjacent to the forelimb representation (within 0.3 - 1.0 mm), but from which no movement could be evoked even at high stimulation currents (60-80 μA). Injections placed into whisker regions exhibiting novel forelimb activity gave rise to large numbers of labeled axons and terminal boutons which extended back into the whisker representation as well as ones which crossed the forelimb/whisker border and extended several mm within the forelimb representation. In contrast, injections placed into whisker regions close to the border but from which no activity could be evoked gave rise to labeled axons and terminal boutons which remained mostly restricted to the whisker representation, with few or no axons that crossed the forelimb/whisker border. These data support the hypothesis that the occurrence and extent of motor map plasticity may be constrained by the pattern of intrinsic connections which exist between adjacent representations. (Supported by grants from the Aaron Diamond Foundation and the Sinsheimer Foundation).

747.7

NMDA RECEPTOR ANTAGONISTS REVERSIBLY BLOCK PLASTICITY OF ADULT MOTOR CORTEX REPRESENTATIONS. G.W. Huntley1*, F. Liang2, T.M. Woods2, J.W. Tullai1, and E.G. Jones2. 1 Fishberg Res. Ctr. for Neurobiology, Mt. Sinai Sch. of Med., New York, NY 10029; 2 Dept. of Anatomy & Neurobiology, University of California, Irvine, CA 92717.

NMDA receptors have been strongly implicated in forms of synaptic plasticity such as LTP, but any role they may play in the mechanisms contributing to activity-dependent changes in neocortical representational maps is unknown. To investigate the role of NMDA receptors in motor cortex plasticity, we utilized a previously described paradigm (Sanes et al., PNAS 85;1988) in which transection of the facial nerve induces a rapid expansion of the cortical forelimb representation into the former whisker representation. The motor cortices of adult rats were first mapped using low-threshold electrical stimulation techniques to evoke movements, then exposed to antagonists (D-APV or MK-801) via slow-release from the polymer Elvax (DuPont) implanted subdurally. After a 2-7 day exposure period, the contralateral facial nerve was cut, and 2-4 hours later the Elvax was removed and the cortex was immediately re-mapped. Forelimb movements were evoked reliably from all previously mapped forelimb positions at comparable thresholds, but no movements could be evoked from the previously mapped whisker region even at high stimulation currents (60-80 μA). After a 4 hour saline-drip to wash out the drug, the motor cortex was again re-mapped, at which time low-threshold forelimb movements were evoked from all previous forelimb positions as well as new spots corresponding to regions of the former whisker representation. The current thresholds were not statistically different from those obtained in the first mapping. Motor cortices exposed to control solutions (saline or the inactive stereoisomer L-APV) exhibited a forelimb border shift at the first mapping immediately after facial nerve cut. These data suggest that NMDA receptors may be a critical component of the synaptic connections which underlie rapid changes in cortical representational maps, and their functional activation may emerge under conditions in which plasticity is induced. (Supported by grants from the Aaron Diamond Foundation, the Sinsheimer Foundation and NS21377).

747.8

DIFFERENTIAL EFFECTS OF SKILL ACQUISITION AND MOTOR USE ON THE REORGANIZATION OF MOTOR REPRESENTATIONS IN AREA 4 OF ADULT SQUIRREL MONKEYS. E.J. Plautz*, G.W. Milliken, & R.J. Nudo. Dept. of Neurobiology & Anatomy, Univ. of Texas Medical School, Houston, TX 77225.

Previous research has shown that representations in primary motor cortex (area 4) are alterable by behavioral experience (e.g.: Milliken, et al., 1992; Nudo, et al., in press). In the present study, we attempted to dissociate effects of *motor skill acquisition* from effects of *motor use*. Intracortical microstimulation techniques were used to derive detailed maps of movement representations of the hand, wrist, and arm in area 4 before and after two types of motor training.

Monkeys were trained to retrieve food pellets from either a small well (9.5 mm diameter) or a large well (19 mm diameter) in a Plexiglas board (Klüver board). Motor performance was assessed by: a) average number of digit flexions per pellet retrieval and b) frame-by-frame video analysis of movements and movement combinations made during pellet retrieval. Training continued until a criterion number of total flexions had been performed. Monkeys trained on the small well displayed poor performance initially but rapidly improved during the training period. Improvement in behavioral performance was paralleled by a change in movements used during pellet retrieval. In contrast, monkeys trained on the large well exhibited nearly flawless retrieval performance throughout the training period and little variation in retrieval movements. A third group of control subjects were not exposed to the training task.

Following small well training, post-training maps revealed areal expansions in the representations of digit movements and digit/wrist combination movements. These expansions closely paralleled the movements used during successful task performance. In contrast, no systematic changes were found in digit or wrist representations following large well training, despite an equivalent number of total digit flexions. Similarly, control subjects showed little variation in movement representations between mapping sessions. These findings suggest that the reorganization of motor representations in area 4 reflects the *acquisition of new motor skills*, and does *not* simply reflect changes in *motor use*.

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747.9

RECOVERY OF FINGER MOVEMENT REPRESENTATION AFTER DISTAL FORELIMB RESTRICTION IN ADULT SQUIRREL MONKEYS. G.W. Milliken*, E.J. Plautz, & R.J Nudo. Department of Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77225.

Using intracortical microstimulation techniques, detailed maps of movement representations in primary motor cortex (area 4) were derived before, during, and after restriction of the preferred distal forelimb. Consequences of distal forelimb restriction on the functional topography of motor cortex involved *decreases* in finger and *increases* in wrist representation (Milliken, et al., 1994, *Soc. Neurosci. Abs., 20,* 1394).

In the present study, we examined behavioral and neurophysiological correlates to motor recovery following removal of forelimb restriction. Behavioral recovery was evaluated using a Klüver board pellet retrieval task. By 78 days, 2 of the 3 subjects had returned to use of their previously restricted and preferred (PRP) hand. Subsequent motor mapping experiments revealed that for these 2 subjects the area of the restriction-diminished finger representations had increased substantially and approximated the baseline finger area. The third subject did not display use of his PRP hand and showed no finger representation recovery at 78 and 161 days post restriction. To force this subject to use his PRP hand, we restricted the contralateral forelimb and generated a motor map after 57 days of use. Forced rehabilitation caused recovery of 86% of the finger representation.

These results indicate that recovery of finger representation in primary motor cortex is related to behavioral use of the PRP hand, and that recovery can occur with forced rehabilitation.

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747.10

LIMITED MOTOR CORTEX REORGANIZATION IN A LONG-TERM MONKEY AMPUTEE. M. H. Schieber[1]*, T. W. Anderson[2] and R. K. Deuel[2]. Departments of Neurology and of Neurobiology, [1]University of Rochester, Rochester, NY, 14642 and [2]Washington University, St. Louis, MO, 63110.

After forelimb amputation in neonatal rats, the primary motor cortex (M1) reorganizes such that stimulation in the previous forelimb region can evoke movements of the face (Donoghue & Sanes, 1988). But in adult rat or human amputees, stimulation of the M1 upper extremity region evokes only enhanced contractions of remaining shoulder stump muscles (Sanes et al., 1990; Cohen et al., 1991). We studied M1 in a 15 year old monkey that for unrelated reasons prior to age 2 had undergone amputation of the right arm at the shoulder joint.

We used intracortical microstimulation (ICMS) to explore the region of M1 normally devoted to the arm and hand bilaterally, and to identify its borders with the face region laterally and the leg region medially. In the right M1, conventional ICMS evoked movements of the left face, arm and leg at current thresholds at or below 40μA; the locations of the face/arm and arm/leg borders were normal. In the left M1 contralateral to the amputated right arm, ICMS similarly evoked movements of the right face and leg from typical locations at normal current thresholds. But within the left M1 arm region, ICMS failed to evoke movements of body parts other than the remaining right shoulder stump. Modified ICMS using currents up to 80μA and trains up to 200ms also failed to evoke movements of the right face, neck, abdomen or leg, or of the left arm.

The M1 arm region contralateral to this monkey's amputation was not reorganized to represent the face or leg. Instead, shoulder stump movements were evoked throughout the arm region, which may have resulted from unmasking of the normal widespread shoulder representation. Species, age at amputation, and/or time since amputation, may affect M1's reorganization.

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747.11

LEARNING AND RETENTION OF A NEOCORTICALLY-DEPENDENT SENSORIMOTOR SKILL IN THE RAT. Michael Coogan*, John Larson, & Gary Lynch. CNLM, Univ. Calif., Irvine, CA 92717.

Traditional models of cortical control of fine, distal limb movements have typically been limited to primate species. However, rats can be trained to use their forepaws to grasp and retrieve small food pellets, and this behavior is similar to the manipulative behaviors of primates. Neuroanatomical and behavioral studies support the notion that rats can serve as a model of primate motor skill learning and performance, and that the normal and pathological reaching behaviors of rats are likely to be directly applicable to the understanding of human cortical pathophysiology. The present experiments describe a paradigm for examining the neural substrates of forelimb reaching behavior in rats.

Adult, male Sprague-Dawley rats were trained to reach for food rewards in an apparatus originally designed by Montoya et al. (1991). This apparatus provides for varying degrees of difficulty in reaching skill. Rats were given a single training trial each day. Analysis of group data suggests that acquisition of this skill is qualitatively and quantitatively similar to acquisition of sensorimotor skills in humans. Analysis of individual learning curves revealed that acquisition of the task consists of three phases, each associated with a plateau of performance. Once acquired, the skill is retained for at least three weeks without practice.

Unilateral sensorimotor cortex ablations profoundly impair performance on this task and, while some recovery occurs, there is a residual deficit in motor control of the contralateral forepaw that is stable for at least five weeks. Videoanalysis demonstrates separate sensory and motor impairments in lesioned rats that are qualitatively similar to those seen in humans following traumatic neocortical damage. Methodological advantages of this paradigm will be discussed, and further applications of this model to the study of human clinical syndromes will be proposed. (Supported by AFOSR, ONR, and NIH).

BASAL GANGLIA: STRIATAL SYSTEMS

748.1

LONGTERM CORTICAL AND STRIATAL SLICE CO-CULTURES: ANATOMY AND ELECTROPHYSIOLOGY OF IDENTIFIED NEURONAL TYPES. B.Teng, D. Plenz, Y. Kuga* and S.T. Kitai, Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

To study cortico-striatal dynamics an *in vitro* system has been developed using organotypic cortex-striatum co-cultures. Previous work showed that the spontaneous activity developing in this system proves to be highly similar to the ones found *in vivo*. Here we further examine the electrophysiological and morphological properties of neurons in longterm (> 4 weeks) co-cultures. Furthermore, as local interneurons were found to profoundly influence cortical and striatal dynamics, a special effort was undertaken to reconstruct those interneurons.

Cortical and striatal slices from rat P0 - P1 were cultured for four to eight weeks using a modified roller-tube technique. Neurons were intracellularly recorded from, electrophysiologically identified and labeled with neurobiotin.

In the cortex, pyramidal neurons, bipolar and multipolar interneurons show similar dendritic and axonal morphologies as *in vivo*. In the striatum, the giant aspiny interneuron class, two GABAergic interneuron classes and medium-spiny projection neurons can be clearly distinguished from each other and are similar to *in vivo*. These results indicate a high degree of similarity in the electrophysiology and morphology between neurons in longterm slice co-cultures and *in vivo*.

Supported by 'Deutsche Forschungsgemeinschaft' and USPHS grant NS 20702.

748.2

CORTEX AND STRIATUM SLICE CO-CULTURES: POPULATION ACTIVITY UNDERLIES HIGH FREQUENCY SUBTHRESHOLD OSCILLATIONS WHICH GOVERN SPIKE DISCHARGE. D. Plenz and S.T. Kitai*. Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

It has been hypothesized that a brief synchronization of spike discharge among a group of neurons is one of the basis for neuronal coding and among cortical neurons has been observed to be ~40 Hz.

In order to study cortico-striatal information processing and the underlying mechanisms of synchronized spike discharge, we have established longterm (4 - 8 weeks) organotypic cortex-striatum co-cultures from rats at age P0 - P1. Simultaneous intracellular recordings were obtained from neurons in the cortex, the striatum or both. Neurons were identified based on their electrophysiological characteristics and their morphological features were revealed by intracellular labeling with neurobiotin.

Findings indicate: (1) In the cortex during periods of spontaneous activity, brief (0.5 - 1.5 s) ~40 Hz subthreshold oscillations were observed to which pyramidal neuron spike discharge is temporally linked. (2) In the striatum, medium-spiny neuron spike discharge is temporally linked to > 20 Hz subthreshold oscillations. (3) In both structures, spike discharge of GABAergic interneurons was closely associated with subthreshold oscillation frequencies.

These results indicate that: (1) The ~40 Hz subthreshold oscillation in the cortex is an emergent property of the cortical network itself. (2) Burst activities (~20 Hz) of striatal medium-spiny projection neurons may be governed by the striatal network, which is driven by cortical inputs. (3) Subthreshold oscillation frequencies in each structure result from population activity in which local GABAergic interneurons strongly participate.

Supported by 'Deutsche Forschungsgemeinschaft' and USPHS grant NS 20702.

748.3

Electrophysiological Characterization of Immunocytochemically Identified Neurons in the Rat Substantia Nigra Pars Reticulata *In Vitro*. C.D. Richards, T. Shiroyama* and S.T. Kitai. Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

Previous studies on the substantia nigra pars reticulata (SNr) have identified two groups of neurons based upon their electrophysiological characteristics. However, the transmitter phenotype of each of these two neuronal classes is unknown. The aim of this study was therefore to record from neurons in the SNr and characterize them electrophysiologically, and subsequently to identify these neurons using immunocytochemical double labelling techniques.

Intracellular recordings were performed in slices of midbrain containing the SNr, prepared using standard techniques. Electrodes contained neurobiotin, which was injected into neurons subsequent to electrophysiological characterization. Two electrophysiological classes of neurons were identified. One had a slow rate of action potential discharge, long duration action potentials, and prominent anomalous inward (I_h) and transient outward (I_A) rectification. The other had a faster rate of action potential discharge, shorter duration action potentials, weak I_h and no apparent I_A.

Both types of neuron were subsequently processed immunocytochemically to reveal the injected biocytin. The former type of neuron, which had electrophysiological characteristics similar to substantia nigra pars compacta neurons, displayed double labelling for biocytin and tyrosine hydroxylase, indicating that they are dopaminergic. The latter, faster firing type of neuron is currently being processed immunocytochemically to determine the presence or absence of glutamic acid decarboxylase (GAD), the marker enzyme for GABAergic neurons.

This study indicates that the SNr contains two classes of neuron which can be distinguished using intracellular recording and electrophysiological criteria.

Supported by USPHS grants NS 20702 and NS 26473.

748.4

A FURTHER CHARACTERIZATION OF RAT TEGMENTAL PEDUNCULOPONTINE (PPN) CHOLINERGIC NEURONS. K. Takakusaki*, T. Shiroyama and S.T. Kitai. Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

We have demonstrated that PPN Type II neurons are cholinergic and characterized by a transient outward current in *in vitro* slice preparations (Kang and Kitai, *Brain Res.* 1990). In this study, Type II neurons (n=69) were classified into 2 subgroups according to their electrical membrane characteristics. One group (short-duration neurons; SDNs, n=21) was characterized by short duration spikes (0.7-1.5 ms), a high spontaneous firing rate (m=14 Hz) and high input resistance (m=230 MΩ). Depolarizing current injection increased the firing rate of these neurons with no alterations in duration or fast-AHP of spikes. The other group (long-duration neurons; LDNs, n=48) was characterized by long duration spikes (1.6-2.8 ms), a low firing rate (m=7.8 Hz) and low input resistance (m=140 MΩ). They displayed accommodation of firing frequency with changes in spike duration and fast-AHP. In the presence of TTX (<10 μM), SDNs displayed Ca-dependent high threshold oscillations (HTO) with high frequency (m=13.5 c/s) and low voltage (m=2.8 mV). LDNs had low frequency (m=7.5 c/s) and large amplitude (m=5.2 mV) HTO. An application of TEA (5 mM) generated spontaneous high threshold Ca-spikes in LDNs but not in SDNs. Biocytin injection combined with ChAT immunohistochemistry revealed 60% of each subtype of neurons were cholinergic. Morphologically, SDNs had small cell bodies (<20 μm) with 2-4 thin primary dendrites and LDNs large cell bodies (>25 μm) with 3-6 thick primary dendrites. Both groups of cells were intermingled within PPN. These results suggest that PPN cholinergic neurons are composed of heterogeneous subgroups of neurons with different membrane characteristics and morphology. *Supported by USPHS grants NS20702 and NS26473.*

748.5

CORTEX-STRIATUM-MESENCEPHALON SLICE CULTURES AS A MODEL FOR ANALYSIS OF FOREBRAIN DYNAMICS. S.T. Kitai, D. Plenz and E.F. Johnson*, Department of Anatomy and Neurobiology, University of Tennessee, College of Medicine, Memphis, TN 38163.

Dopamine influences cortical and striatal activity at the single neuron level as well as at the neural network level. In previous work it has been shown that the spontaneous network activity developing in organotypic cortex-striatum co-cultures is highly similar to the ones found *in vivo*. Here we extend this *in vitro* system analysis of cortical-striatal dynamics by including dopaminergic projection neurons from the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA).

Cortical, striatal and mesencephalic (SNc/VTA) slices from rat P0 - P1 were cultured for up to 8 weeks using a modified roller-tube technique. Triple-cultures were stained for tyrosine hydroxylase (TH) immunoreactivity.

After three weeks in culture TH-positive neurons were found in the mesencephalic tissue region. A high abundance of TH-positive fibers are present in the cortex and the sriatum. Intracellular recordings from 7 week old striatal neurons show that they respond to SNc/VTA, cortical and intrastriatal stimulation, each with unique features.

We propose that the triple-culture will provide a powerful *in vitro* approach to study the role of dopaminergic systems in cortico-striatal network dynamics.

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748.6

SOMA-DENDRITIC DOPAMINERGIC (DA) MODULATION OF ACTIVATED PREFRONTAL CORTEX→NUCLEUS ACCUMBENS (PFC→NAC) NEURONS *IN VITRO*. Gorelova, N., Yang, C.R., & Seamans, J.K. Dept. of Psychology, & Psychiatry, Univ. of British Columbia, Vancouver, CANADA,V6T 1Z4.

Corticostriatal pyramidal neurons in deep layers V-VI of the PFC link the NAC and receive the mesocortical DA input. This PFC→NAC pathway may be an important route via which DA modulation of PFC signals encoding for cognitive processes is translated into actions. To evaluate the underlying mechanisms of DA modulation in PFC→NAC neurons, we have made biocytin-intracellular recordings in: a) antidromically activated PFC neurons that responded to NAC stimulations in brain slices, and b) PFC slices prepared from rat which received prior microinjection of the retrograde tracer rhodamine beads in the NAC.

Bath applications of DA D1 agonist (SKF38393;10-50μM), but not D2 agonist (qunpirole), enhanced the excitability of intrinsic bursting PFC→NAC neurons in response to depolarizing pulses via its interactions with two opposing voltage-dependent currents: 1) a direct *suppression* of a dendrotoxin (50nM)- and 4AP(0.1-2mM)-sensitive, outwardly rectifying, slowly inactivating K^+ current, but 2) a direct *augmentation* of a somatic, TTX-sensitive, inwardly rectifying, slowly inactivating Na^+ current.

On the other hand, the regenerative dendritic high-threshold Ca^{2+} spikes (HTS) (in TTX+TEA) or the Ba^{2+} plateau potentials (recorded when $[Ca^{2+}]_o$ were substituted by Ba^{2+} to prevent $[Ca^{2+}]_o$ auto-inhibition of HTS) were suppressed markedly by SKF38393. Furthermore, D2 receptor stimulations by quinpirole (50μM) following glutamate receptor blockade (by 10μM CNQX and 50μM APV) increased transiently the frequency of GABAergic IPSPs. Hence, D1 receptor activation augments input signals reaching the deep layer somatic region via its interactions with the slow K^+ and Na^+ currents. D1 receptor stimulation also suppresses the overall HTS-amplification of associative input signals to the apical dendrites (in layers I-II) of the deep layers V-VI PFC→NAC neurons. In addition, D2 receptor activation of the GABAergic interneurons attenuated the excitability of the PFC→NAc neurons. (Funded by B.C.Health Research Foundation & MRC of Canada)

748.7

DOPAMINE (DA) MODULATION OF POST-SYNAPTIC RESPONSES IN LAYER V-VI RAT PREFRONTAL CORTEX (PFC) NEURONS EVOKED BY SYNAPTIC ACTIVATION OF THEIR APICAL DENDRITES *IN VITRO*. C. R.Yang* & J.K. Seamans, Dept.of Psychology, & Psychiary, Univiversity of British Columbia, Vancouver, B.C., V6T 1Z4

Activation of NMDA receptors in the dendrites of pyramidal neurons promotes Ca^{2+} influx through dendritic NMDA receptors and high-voltage activated Ca^{2+} channels. We have previously shown *(Yang and Seamans, Soc. Neurosci. Abst*, 1994) that DA D1 receptor stimulation suppressed dendritic high threshold Ca^{2+} spikes (HTS) evoked by intracellular depolarizing current pulses in PFC cells. To determine the functional interactions of D1 receptor with HTS arising from synaptically-evoked, NMDA receptor-mediated EPSPs, the effects of the D1 agonist (SKF38393) and antagonist (SCH23390) were studied in intracellularly recorded PFC neurons in rat brain slices (QX-314 (100mM)-filled electrodes in bicuculline (10μM), CNQX(10μM), V_m held at -60mV).

Lower frequency (0.02-0.1Hz) electrical stimulation of the superficial layers I-II, where the apical dendrites of the layer V-VI neurons are located, synaptically evoked an early NMDA-receptor mediated EPSP, followed by a late, low amplitude, Ca^{2+}-mediated component. This late component developed into an HTS at higher stimulation intensities. Bath-application of SKF-38393 (30-60μM) reduced, while SCH-23390 augmented, the duration of the late Ca^{2+} components. Higher frequency stimulation (>0.2Hz) markedly attenuated the late Ca^{2+} components perhaps due to an inactivation of the voltage-gated Ca^{2+} channels by the elevated $[Ca^{2+}]_i$ under this condition At high frequency stimulations (≥1Hz), only the initial NMDA component persisted and SCH-23390 (20-50μM) reversibly attenuated (28%) the amplitude of this isolated NMDA component. These data suggest that synaptically-released DA activates D1 receptors to potentiate NMDA signals. Additional DA suppression of the overall dendritic signal amplification mediated by the synaptically-evoked dendritic Ca^{2+}-HTS results in a selective "sharpening" of the potentiated NMDA signals. (Funded by B.C.H.R.F. & M.R.C.of Canada).

748.8

D1 DOPAMINE RECEPTOR-INDUCED IMMEDIATE EARLY GENE EXPRESSION IN THE DOPAMINE-DEPLETED STRIATUM IS LARGELY INDEPENDENT OF EXCITATORY AFFERENT INPUT AND ACTION POTENTIALS. K.A. Keefe* and C.R. Gerfen, Laboratory of Systems Neuroscience, NIMH, Bethesda, MD 20892

Cortical input to striatum produces excitatory post-synaptic potentials and action potentials in striatal efferent neurons mediated primarily by the AMPA subtype of glutamate receptor. Activation of D1 dopamine receptors modifies the response of striatal neurons to cortical input. Although expression of immediate early genes often is used as a marker of neuronal activity, the extent to which D1-mediated induction of immediate early genes in striatum reflects enhanced neuronal activity to excitatory input remains unclear. We therefore examined the effects of intrastriatal administration, via *in vivo* microdialysis probes, of CNQX or TTX on induction of immediate early genes by D1 receptor stimulation in dopamine-depleted rats. Local infusion of AMPA into striatum induced c-*fos* and *zif268* throughout striatum and, in some cases, throughout the ipsilateral cerebral cortex. Co-infusion of CNQX with AMPA blocked the induction, suggesting that CNQX effectively blocks AMPA receptors. However, infusion of CNQX (10 μM - 1 mM) did not alter induction of *zif268* or c-*fos* by SKF 38393, except at the highest concentration where variable effects on c-*fos* expression were observed. Similarly, when SKF 38393 was injected during perfusion of striatum with TTX, induction of *zif268* was not affected. Expression of c-*fos* showed a small (23%), but significant decrease. These data suggest that induction of *zif268* and c-*fos* by SKF 38393 in the dopamine-depleted striatum is largely independent of input through the AMPA subtype of glutamate receptor and action potential activity in striatum.

748.9

ENKEPHALIN INHIBITS IMMEDIATE-EARLY GENE EXPRESSION INDUCED BY BLOCKADE OF D2 DOPAMINE RECEPTORS IN STRIATUM. H. Steiner*, R. Mariotti and C. R. Gerfen. Laboratory of Systems Neuroscience, NIMH, Bethesda, MD 20892.

Recent studies showed that dynorphin, an opioid peptide contained in striatonigral projection neurons, regulates dopamine input to these neurons. This opioid-dopamine interaction was demonstrated by using induction of immediate-early genes (IEGs) by dopamine receptor stimulation as a functional marker. Striatal neurons that project to the globus pallidus express D2 dopamine receptors and contain the opioid peptide enkephalin. In the present study, we investigated whether enkephalin has an autoregulatory function in striatopallidal neurons similar to dynorphin in striatonigral neurons. Thus, we examined whether enkephalin or agonists inhibit IEG induction by a D2 dopamine receptor antagonist in striatopallidal neurons. Gene expression was assessed by quantitative *in situ* hybridization histochemistry. Acute injection of the D2 antagonist eticlopride results in rapid induction of the IEGs c-*fos* and *zif 268*. Repeated treatment (1x/day, 1-5 days) with the D2 antagonist produced an increase in expression of enkephalin that was paralleled by suppressed inducibility of IEGs. Moreover, infusion of the delta opioid receptor agonist DADLE (0.05-5 nmol) into the striatum suppressed IEG induction by acute eticlopride. These results suggest that enkephalin acts in the striatum to blunt activation of striatopallidal neurons by D2 blockade and that increased enkephalin expression after repeated D2 antagonist treatment is a compensatory response to limit overactivation.

748.10

SYSTEMIC BUT NOT INTRASTRIATAL INJECTION OF THE MUSCARINIC AGONIST, OXOTREMORINE, INDUCES CORTICAL AND STRIATAL IMMEDIATE EARLY GENE EXPRESSION. L.K. Nisenbaum*, S.T. Kitai, and C.R. Gerfen, Dept. of Anatomy & Neurobiology, Univ. of Tennessee, College of Medicine, Memphis, TN, and Section on Neuroanatomy, NIMH, Bethesda, MD.

Acetylcholine and dopamine have been hypothesized to interact within the striatum to influence the output neurons of this structure. Both dopaminergic agonists and muscarinic antagonists alter the expression of enkephalin and substance P mRNA expression in the dopamine-depleted striatum. We have previously demonstrated that whereas local stimulation of dopamine receptors mimics the effect of systemic drug administration, striatal infusion of a muscarinic antagonist does not alter striatal peptide gene expression.

To examine the role of muscarinic receptor activation in immediate early gene regulation, we have used *in situ* hybridization histochemistry to measure the effect of systemic and intrastriatal muscarinic drug administration in normal and 6-hydroxydopamine- (6-OHDA) lesioned rats. Systemic injection of oxotremorine (0.5 mg/kg, i.p.) produced an increase in cortical and striatal *c-fos* and *zif268* expression in normal animals. In 6-OHDA-lesioned rats, the induction of *c-fos* and *zif268* by systemic oxotremorine was significantly reduced. In contrast, intrastriatal infusion of oxotremorine (50 pmol in 0.5 μl) did not increase *c-fos* or *zif268* expression in either the striatum or the cortex of normal or 6-OHDA-lesioned rats. To examine the tonic influence of muscarinic receptor activation on immediate early gene expression, the effect of systemic and intrastriatal administration of the muscarinic antagonist, scopolamine, was tested. No effect of scopolamine was observed on *c-fos* or *zif268* expression in control or 6-OHDA-lesioned rats. These data demonstrate that extrastriatal activation of muscarinic receptors is responsible for the induction of immediate early genes by oxotremorine. In addition, the lack of effect by either systemic or intrastriatal scopolamine administration suggests that immediate early gene expression is not tonically regulated by acetylcholine. *Supported by USPHS grants NS26473, NS09362.*

748.11

STRIATAL EXPRESSION OF *ZIF* 268 mRNA TO ACUTE 5HT-2A AND 5HT-2C RECEPTOR STIMULATION AFTER UNILATERAL LESIONING OF THE NIGROSTRIATAL PATHWAY. R. F. Paletzki* and C. R. Gerfen. Laboratory of Systems Neuroscience, NIMH, Bethesda, MD 20892.

The striatum receives a dopamine projection form the substantia nigra and a serotonin projection from the dorsal raphe. Whereas dopamine differentially modulates the function of connectionally distinct striatal neurons, the influence of serotonin on striatal neurons is less understood. In this study the effect of serotonin, via the 5HT-2A and 5HT-2C receptors, on striatal neuron function was investigated in the presence and absence of endogenous dopamine input. Rats were given a single injection of either ±DOI or mCPP (0,1,10 mg/kg) 3 weeks following unilateral lesion of the dopaminergic nigrostriatal pathway. Alterations in striatal neuron function were determined by measuring changes in expression of the immediate early gene *zif* 268 mRNA by *in situ* hybridization with ^{35}S-labeled oligonucleotide probes. ±DOI, which has higher affinity for 5HT-2A over 5HT-2C receptors, increased expression of *zif* 268 in the lesioned striatum, while producing smaller changes in the intact striatum. mCPP, which has greater affinity for the 5HT-2C receptor, significantly reduced *zif* 268 mRNA expression in the lesioned striatum, but had little effect on the intact side. These data indicate that serotonin differentially affects striatal neuron function via the 5HT-2A and 5HT-2C receptors. The 5HT-2A receptor appears to increase striatal neuron function, whereas the 5HT-2C receptor appears to decrease striatal neuron function. Furthermore, these data demonstrate that dopamine can reduce the response of striatal neurons to serotonin receptor stimulation.

748.12

GAP-43 EXPRESSION IN THE STRIATUM AFTER THERMOCOAGULATORY AND ASPIRATION LESIONS OF THE SENSORY-MOTOR CORTEX: AN ULTRASTRUCTURAL STUDY. Kunihiro Uryu*, Larami MacKenzie and Marie-Françoise Chesselet, Dept of Pharmacology, University of Pennsylvania , Philadelphia, PA 19104 USA

We have previously reported (Szele et al. , J. Neurosci., in press) that expression of GAP-43, an axonal protein enriched in growth cones and sprouting axons, is decreased in the striatum after unilateral lesions of the sensory-motor cortex induced by acute aspiration, but not after progressive lesions induced by thermocoagulation of pial blood vessels. We have examined GAP-43 immunostaining at the ultrastructural level in the striatum of rats 16 days after both types of lesions. Both lesions induced similar loss of all layers of the sensory-motor cortex . In control rats, GAP-43 immunoreactivity in the dorsolateral striatum was most often associated with axon terminals enriched in varied-sized clear vesicles (Growth cone-like structures). These processes occasionally formed asymmetric contacts with dendritic spines. GAP-43 immunoreactivity was also present in pre- and terminal segments of axons, and to a lesser extend in axon terminals forming asymmetrical and symmetrical synapses and in dendritic spines. After aspiration lesions, extensive degeneration was present in the denervated dorso-lateral striatum; GAP-43 immunoreactivity was undetectable, and growth cone-like axon terminals were absent. In contrast, expression of GAP-43 and growth cone like axon terminals similar to those seen in controls were present in the dorsolateral striatum after thermocoagulatory cortical lesions. Taken together with data showing increased labeling of crossed corticostriatal projections after thermocoagulatory lesions (Napieralski et al., this meeting), the data suggest that compensatory axonal sprouting occurs in the dorsolateral striatum after thermocoagulatory but not aspiration cortical lesions. Sup. by NS29230.

748.13

A ROLE FOR CONTRALATERAL CORTICOSTRIATAL NEURONS IN THE EFFECTS OF THERMOCOAGULATORY LESIONS OF THE CORTEX ON STRIATAL GENE EXPRESSION. J.A. Napieralski*, A.K. Butler, and M-F. Chesselet, Dept. of Pharmacology, Univ. of Penn., Phila, PA 10104

Previous studies in our laboratory have shown that cortical lesions induced by thermocoagulation of pial blood vessels, but not by acute aspiration, result in 1) the preservation of control levels of GAP-43 and 2) a prolonged increase in neurotransmitter gene expression in the denervated dorsolateral striatum. We have examined whether corticostriatal projections from the spared homotypic contralateral cortex contribute to these effects. Adult rats received a unilateral lesion of the cerebral cortex and, after thirty days, received an injection of the anterograde tracer, Ruby red, in the contralateral homotypic cortex. Rats were sacrificed 7 days later and labeled fibers were examined with fluorescence microscopy in the ipsilateral and contralateral striata. Ipsilateral corticostriatal projections were labeled in lesioned and unlesioned rats. Numerous labeled fibers were detected in the contralateral striatum of lesioned but not control animals, suggesting that contralateral cortical neurons may undergo axonal sprouting in the denervated striatum. To determine whether contralateral corticostriatal fibers play a role in the changes in gene expression in the striatum induced by the cortical lesion, the effects of unilateral and bilateral thermocoagulatory lesions of the cortex were compared. In situ hybridization histochemistry revealed an increase in the enkephalin mRNA levels in the striatum of unilaterally but not bilaterally lesioned animals, suggesting that sprouting or over activity of contralateral corticostriatal input is responsible for the increase seen after unilateral lesions. Supported by PHS grants NS-29130 and MH10794.

748.14

TOPOGRAPHICAL PATTERNS OF EFFERENTS FROM THE 'BARREL' CORTEX TO RAT NEOSTRIATUM. A.K. Wright, E.A.M. Hutton, L. Norrie, C.A. Ingham, G.W. Arbuthnott.*, *MRC External Scientific Staff, University of Edinburgh Centre for Neuroscience, Preclinical Veterinary Sciences, Summerhall, Edinburgh, EH9 1QH.*

Individual whiskers on the face of rats are represented by an area of S1 cerebral cortex called a 'barrel'. Individual barrels, identified by cytochrome oxidase histochemistry, have been injected in pairs with different anterograde tracers and the projections of neurones within single barrels into the neostriatum, thalamus, and brain stem, examined.

We have used biotinylated dextran amine (BDA) and Phaseolus Vulgaris Leucoagglutinin (PhaL) to fill adjacent barrels in the same rat and after two weeks survival time have stained the two tracers different colours with 'Vector' chromagens DAB and SGA. The axons from the injected cells can be followed into the internal capsule from which they exit at right angles to supply small areas within the somatosensory field of the neostriatum. Axons continue within the internal capsule fibres to other areas of brain including thalamus and brain stem.

There is a topographically arranged pattern of boutons in the dorsolateral portion of the striatum with very little overlap between fibres from individual barrels. Barrels of row A are closest to the callosum while those of row E are deepest in the striatum. A pattern of finer terminals is also visible which may have a different source within the barrel cortex and which seems to have a different topography.

Electron microscopic investigation shows both large boutons making complex synapses with dendritic spines and smaller fibres making simple synapses with individual spine heads. In view of the likely loss of synapses of the asymmetric type when dopamine is lost from the striatum (Ingham et al (1993) Exp.Br.Res. 93 17) it will be important to compare the fate of these synapses after 6-hydroxydopamine treatment.

BASAL GANGLIA: DRUGS OF ABUSE

749.1

COCAINE AND AMPHETAMINE FAIL TO INDUCE C-FOS- AND JUN B-LIKE PROTEINS IN THE STRIATUM OF DOPAMINE D1 RECEPTOR MUTANT MICE . R.Moratalla[1]*, M.Xu[2], S.Tonegawa[2] and A.M. Graybiel[1].. [1]Dept. Brain & Cog. Sci.,[2]Ctr. for Cancer Research, MIT, Cambridge, MA 02139.

Cocaine and amphetamine are psychomotor stimulants that exert powerful acute and long-term effects on behavior. A candidate mechanism for mediating the long-term effects is the induction of cascades of regulation, including regulation of immediate-early genes (IEGs) coding transcription factors and genes coding neuromodulators such as dynorphin in the basal ganglia and associated circuitry. Pharmacological experiments have implicated D1-class dopamine receptors in many of these effects, but actions of other receptors (e.g. dopamine D2-class and 5HT) have also been suggested. We previously reported experiments (Xu et al 1994) showing that D1 mutant mice have severe deficits in dynorphin (and substance P) in the striatum and associated basal ganglia nuclei and exhibit diminished behavioral responses to acute cocaine. We have now tested whether, in such D1 mutants, acute cocaine and amphetamine can induce IEGs in the striatum. Wildtype (wt) and mutant mice were given cocaine (40 mg/kg) or amphetamine (10 mg/kg) under single-blind conditions, and 2 hr later their brains were fixed and processed for c-Fos and Jun B immunostaining with polyclonal antisera. Mutant and wt controls were untreated or given haloperidol (2 or 5 mg/kg). The results were clear-cut. Cocaine and amphetamine strongly induced c-Fos- and Jun B-like proteins in the striatum of wt mice in drug-specific patterns (Graybiel et al 1990), but such induction was virtually absent in the D1 mutant mice as were stereotypies and locomotion. Forebrain IEG induction was also reduced elsewere. By contrast, haloperidol induced both IEGs in mutants as well as in wt mice. We conclude that the D1 receptor is essential for striatal induction of c-Fos and Jun B-like proteins by cocaine and amphetamine. Deficits in IEG induction could contribute to the neuropeptide deficiencies in the basal ganglia of these mice and to their behavioral abnormalities as well. Supported by NIDA DA08037.

749.2

WITHDRAWAL FOLLOWING REPEATED AMPHETAMINE TREATMENT INCREASES DYE COUPLING BETWEEN NEURONS AND ACTIVATES OUTPUT PATHWAYS IN RAT LIMBIC CORTICAL/STRIATAL REGIONS S.P. Onn* and A. A. Grace, *Depts Neuroscience & Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260*

Although the mesocorticolimbic dopamine (DA) system has frequently been implicated in behavioral sensitization, its cellular or synaptic mechanisms are unclear. Utilizing *in vivo* intracellular recordings and Lucifer-yellow dye injection, we examined the impact of amphetamine sensitization on dye coupling between dopaminoceptive postsynaptic target neurons in rat striatal and cortical regions. Following amphetamine treatment (2-4 mg/kg; i.p., daily, 1-4 weeks) and 3-7 days of drug withdrawal, significantly higher levels of dye coupling were observed between projection neurons in the infralimbic/piriform cortices (rAMP =5/6 vs basal =1/6) as well as in the ventral striatum (rAMP =13/14 vs basal 2/11). In contrast, no increase in coupling was observed following acute amphetamine (5mg/kg; 0/6). The increased coupling observed following rAMP was similar to that observed following >95% depletions of striatal DA (12/16). Nevertheless, the density of tyrosine hydroxylase (TH)-immunoreactive terminals in the striatum was not substantially altered in the rAMP-treated rats. Thus, these combined data suggest that withdrawal following rAMP produces a functional denervation of the DA system without causing an actual loss of DA terminals. Furthermore, activity within the ventral cortical/striatal output pathways, as revealed by c-fos immunoreactive levels, showed an elevation above control levels in the rats following rAMP withdrawal. (Support by NARSAD Young Investigator Award and NS19608, MH42217 & 45156).

749.3

CANNABINOID AGONIST WIN 55212-2 MODULATES STRIATO-NIGRAL NEUROTRANSMISSION BY A PRESYNAPTIC MECHANISM. T. Tersigni and H.C. Rosenberg,* Dept. of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699.

The brain cannabinoid receptor has been shown to be present in areas receiving striatal innervation, including the pars reticulata of the substantia nigra (SN), and to be located presynaptically on the GABAergic striatal output neurons. This study tested the hypothesis that cannabinoid agonist, applied locally, could modulate striato-nigral transmission, but would not affect the response of the postsynaptic neuron to locally-applied GABA. Male Sprague-Dawley rats were anesthetized with chloral hydrate. A glass multi-barrel electrode was used to record the spontaneous activity of SN neurons, to iontophoretically apply GABA, and to pressure eject Win 55212-2, a cannabinoid agonist, in vehicle. This electrode assembly was lowered into the SN using stereotaxic coordinates, and SN neurons were identified by the firing rate and shape of the action potential. Recording sites were marked for histological confirmation. Win 55212-2 increased the spontaneous neuronal firing rate by 30-46 percent. The peak effect of Win 55212-2 was reached 4.5-8.5 minutes after onset of pressure ejection. The percent inhibition of neuronal activity produced by GABA was not altered in the presence of Win 55212-2, indicating that Win 55212-2 had no effect on the interactions of GABA at the postsynaptic membrane. Pressure ejection of vehicle, 45% 3-hydroxypropyl-β-cyclodextin, produced a small, non-significant decrease in spontaneous neuronal discharge, and no change in response to GABA. These findings suggest that cannabinoid receptors on the striatal terminals can modulate striato-nigral neurotransmission presynaptically. Supported by NIH grant DA 02194.

749.4

PHYSICAL WITHDRAWAL IN RATS TOLERANT TO Δ^9-THC PRECIPITATED BY A CANNABINOID RECEPTOR ANTAGONIST Kang Tsou, Saundra L. Patrick, Russell M. Church* and J.Michael Walker. Schrier Research Laboratory, Departments of Psychology and Neuroscience, Brown University, Providence, RI 02912.

Whereas termination of chronic intake of opiates, barbiturates, and certain other psychoactive drugs leads to serious physical withdrawal symptoms, discontinuation of heavy use of marijuana leads to few if any withdrawal symptoms in animals and humans. This is perhaps due in part to the slow elimination of marijuana's main psychoactive constituent Δ^9-tetrahydrocannabinol (Δ^9-THC). To test the hypothesis that rapid termination of interactions between Δ^9-THC and cannabinoid receptors would lead to withdrawal symptoms in tolerant animals, we precipitated withdrawal with the newly-developed competitive cannabinoid antagonist SR141716A. Tolerance to Δ^9-tetrahydrocannabinol (Δ^9-THC) was produced in rats by twice daily injections (15 mg/kg i.p.) for 6.5 days; a separate group of animals received the vehicle at the same times. One and 1/2 hours following the last injection, animals received an injection of the vehicle or SR141716A. This drug produced few effects in animals that had received previous injections of the vehicle only. By contrast, SR141716A induced a profound precipitated withdrawal syndrome in THC-tolerant animals. The syndrome was characterized by constantly changing brief sequences of behavior resulting in a hyperactive appearance but lacking the sequential organization which remains intact during the hyperactive state produced by psychostimulant drugs. The withdrawal syndrome began about 10 minutes after injection of the antagonist and last for one to two hours. THC-tolerant animals that were treated with vehicle remained quiet throughout the observation period. Administration of the antagonist i.c.v. to tolerant animals produced a milder withdrawal syndrome, suggesting that the site of action of the antagonist for the most profound behavioral changes lies outside the periventricular core of the brain. These findings demonstrate that following long-term use, rapid discontinuation of cannabinoid agonist/receptor interactions leads to profound withdrawal symptoms.

749.5

ELECTROPHYSIOLOGICAL EFFECTS OF A CANNABINOID ON NEURAL ACTIVITY IN THE GLOBUS PALLIDUS. A.S. Miller* and J.M. Walker. Schrier Research Laboratory, Department of Psychology, Brown University, Providence, RI 02912.

The high density of cannabinoid receptors in the globus pallidus and the motor effects of cannabinoids microinjected into the globus pallidus suggest that the globus pallidus might be an important site for the regulation of motor activity by cannabinoids. Extracellular single neuron electrophysiology was used to explore the role of cannabinoids on neural transmission in the globus pallidus. The synthetic cannabinoid WIN 55,212-2 (0.0625 - 0.5 mg/kg, i.v.) dose-dependently decreased the basal firing rate of neurons in the globus pallidus. This effect was not observed following administration of the inactive enantiomer, WIN 55,212-3. A second study examined the effect of WIN 55,212-2 on striatal-stimulation-evoked activity in the globus pallidus. Striatal stimulation produced a brief inhibition of neural activity in the globus pallidus. WIN 55,212-2, but not WIN 55,212-3, reversed the inhibition produced by striatal stimulation. The inhibitory effect of WIN 55,212-2 on the basal activity of neurons in the globus pallidus was apparent in the striatal stimulation study as well. These observations suggest that cannabinoids may produce two different effects on neural activity in the globus pallidus - inhibition of basal activity and a reversal of the inhibition produced by striatal stimulation.

749.6

EFFECT OF INTRANIGRAL ADMINISTRATION OF CANNABINOIDS UPON ROTATIONAL BEHAVIOR: INTERACTION WITH THE DOPAMINERGIC SYSTEM M. Clara Sañudo-Peña, Saundra L. Patrick*, Robert L. Patrick and J. Michael Walker. Schrier Research Laboratory, Departments of Psychology and Neuroscience, Brown University, Providence, RI 02912.

Cannabinoid receptors have been reported to be highly concentrated in brain areas involved in the control of movement, which is in accordance with their well known effects on motor behavior. One of the areas with the highest concentration of cannabinoid receptors is the substantia nigra, which is the output of the basal ganglia for movement production and therefore has been classically used for studying neurotransmitter function in rotational behavior. Cannabinoid receptors in this structure have been shown to be on the striatonigral afferents colocalized with D1 dopamine receptors. The aim of this work was to study the effect of cannabinoids on the rotational behavior induced by unilateral infusion of dopamine agonists into the rat substantia nigra pars reticulata. Cannabinoid compounds have little effect on rotation when injected intranigrally. However, they reduced the contralateral rotation induced by the dopaminergic agonists acting mainly through D1 receptors. These results are in accordance with the opposite action of cannabinoid and D1 receptors on second messengers and neurotransmitter release from striatonigral terminals.

BASAL GANGLIA: ANATOMY

750.1

D1 DOPAMINE RECEPTORS INFLUENCE FOS IMMUNOREACTIVITY IN THE GLOBUS PALLIDUS AND SUBTHALAMIC NUCLEUS OF INTACT AND 6-OHDA-LESIONED RATS. D.N. Ruskin* and J.F. Marshall, Dept. of Psychobiology, University of California, Irvine, 92717.

The globus pallidus (GP) and subthalamic nucleus (STN) are major nuclei of the basal ganglia. Studies of the dopaminergic physiology of these nuclei have typically emphasized the role of the D2 dopamine receptor. However, effects of D1 receptor activation on GP firing rate and STN metabolism have been reported, especially after nigrostriatal lesion. We have systematically investigated the effects of D1 and D2 receptor activation on the activity of the GP and STN in sham-lesioned and unilateral nigrostriatal 6-OHDA-lesioned rats using immunostaining of brain sections for the immediate-early gene Fos. 4-5 wks post-op, rats were drug-injected and perfused 2 hrs later. Brains were sectioned at 40μm and stained with an anti-Fos antibody, visualized with diaminobenzidine. In intact rats, the D1 agonist SKF 38393 (20 mg/kg) produced a five-fold potentiation of the GP Fos due to the D2 agonist quinpirole (0.5 mg/kg), while having no effect alone. In the STN of intact rats, only the combination of SKF 38393 and quinpirole produced significant Fos. In the lesioned hemisphere of 6-OHDA-lesioned rats, SKF 38393 increased the Fos immunostaining in both the GP and STN, while quinpirole increased it only in the GP. SKF 38393 effects in the GP and STN of 6-OHDA-lesioned rats were blocked completely by the D1 antagonist SCH 23390 and unaffected by the D2 antagonist eticlopride, confirming the specificity of the SKF 38393 effect for the D1 receptor. SKF 38393-induced GP Fos after 6-OHDA lesion may be due to increased input from the STN. An excitatory action of dopaminergic agonists on the STN is unexpected given present basal ganglia models, and it is uncertain what circuitry mediates this effect. These results are a novel demonstration of control of Fos expression by dopaminergic drugs in the STN and by D1 agonists in the GP.

750.2

DESCENDING PROJECTIONS OF THE GLOBUS PALLIDUS AND THE VENTRAL PALLIDUM IN THE RAT: CONVERGENCE OF FUNCTIONALLY DIFFERENT PATHWAYS ONTO INDIVIDUAL NEURONS IN THE BASAL GANGLIA. M.D. Bevan*, A.D. Smith and J.P. Bolam MRC Anatomical Neuropharmacology Unit & Department of Pharmacology, Mansfield Road, Oxford, OX1 3TH, U.K.

It has been proposed that one of the functions of the basal ganglia is to act as a site of integration of motor and limbic information. Although it is clear that there are multiple parallel pathways through the basal ganglia, it is not clear where, or indeed, if these pathways converge. In the present tracing and immunocytochemical study of the descending projections of the globus pallidus (GP; which receives sensorimotor-associational inputs) and the ventral pallidum (VP; which receives limbic input) we address this issue. Double anterograde tracing revealed that medial GP and VP innervated largely separate but to some extent overlapping regions of the entopeduncular nucleus/lateral hypothalamus (EP/LH), subthalamic nucleus/lateral hypothalamus (STN/LH) and the substantia nigra pars reticulata (SNr). The GP and VP also projected to the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA). In zones of overlapping termination, the somata of individual neurons were apposed by GP and VP boutons. Single anterograde tracing in combination with retrograde tracing or immunocytochemistry revealed that neurons in the STN/LH that projected to the substantia nigra were apposed by GP or VP boutons. In addition, the midbrain dopaminergic neurons (i.e. tyrosine hydroxylase-immunoreactive) and SNr output neurons (i.e. tyrosine hydroxylase-immunonegative) were apposed by GP or VP boutons. The synaptic organization underlying these patterns of innervation are currently being examined by correlated light and electron microscopy. This analysis indicates that individual neurons in SNr do indeed receive convergent synaptic input from anterogradely labelled GP and VP boutons. We therefore propose that while the basal ganglia clearly process cortical information arising from different functional regions in parallel, some convergence of information occurs at the level of individual neurons (funded by Wellcome Trust and MRC, U.K.)

750.3

EM MORPHOMETRY REVEALS THAT NEUROTENSIN IMMUNOREACTIVITY IS ELICITED IN DIFFERENT SUBPOPULATIONS OF STRIATAL AXONS FOLLOWING DOPAMINE D-2 RECEPTOR ANTAGONIST AND RESERPINE ADMINISTRATION. D.S. Zahm*, E.S. Williams and B. Senger. Dept. of Anat. and Neurobiol., Saint Louis Univ. School of Medicine, St. Louis, Missouri 63104.

Perikaryal neurotensin (NT) immunoreactivity (IR) is undetectable in normal rat striatum, but is elicited following administrations of dopamine D-2 receptor antagonists and reserpine in subpopulations of striatal neurons that differ in terms of sizes and distributions of NT-IR perikarya and fibers (Neurosci., 46:335-350, 1992, 57:649-660, '93 & 65:71-86, '95) and expression of the Fos immediate-early gene product (Neurosci. 57:649-660, '93 & 65:71-86, '95). Whether elicited by D-2 antagonists or reserpine, NT-IR neurons have been shown to project to the globus pallidus and ventral pallidum to the near exclusion of the substantia nigra/VTA complex (Soc. Neurosci Abstr. 19:129, 1993 & Brog and Zahm, unpublished). Rats were given either two injections (2 mg/kg) of haloperidol or eticlopride at four and twenty-four hours prior to sacrifice or a single injection of reserpine (5 mg/kg) twenty-four hours prior to sacrifice and killed by perfusion with buffered aldehydes. Frontal sections were processed for NT-IR and flat embedded for transmission electron microscopy. A random sampling method and computer-assisted morphometric analysis revealed NT-IR bouton profiles that are significantly smaller following reserpine than after haloperidol administration ($p < 0.001$, Mann-Whitney U test). Fewer NT-IR synaptic boutons were observed following haloperidol (1.6% vs 3.0%, $p < 0.012$, paired t-test), probably due in part to the lesser probability of section planes encountering synapses in larger terminals. Synapses were almost exclusively symmetric axodendritic. The EM data are consistent with morphologically distinct subpopulations of striatal neurons that express NT. Supported by USPHS NIH NS-23805 (NINDS).

750.4

REGULATION OF DOPAMINE D_1- AND D_2- RECEPTOR BINDING AND PEK mRNA LEVELS BY SELECTIVE DOPAMINE ANTAGONISTS IN THE BASAL GANGLIA OF THE RAT. A. E. Johnson*, H. Coirini, L. Källström, J. Yu and E.-A. Wiesel. Department of Psychiatry, University Hospital, Uppsala University, S-75017 Uppsala, Sweden.

Chronic treatment with classical neuroleptics affects a number of neurotransmitter systems in the basal ganglia. For example, we have shown that D_1-receptor binding in the EP is enhanced by exposure to a classical neuroleptic. Furthermore, increases in striatal PEK mRNA expression have been reported by several groups. However, the specific dopamine (DA) receptor subtype involved in the regulation of these processes remains to be determined. The purpose of the present study was to examine the regulation of D_1-receptor binding and PEK mRNA levels using selective dopaminergic antagonists. For these studies, adult rats were injected twice daily for 21 days with distilled water, SCH-23390 (0.3mg/kg), Raclopride (0.3mg/kg) or both Raclopride and SCH-23390 (N=6/group). Animals were killed 40hrs after the last injection and brain sections were processed for receptor autoradiographic and in situ hybridization studies. For receptor binding assays, sections were labeled with either [^{125}I]SCH-23982 (D_1) or [^{125}I]NCQ-298 (D_2). An oligonucleotide probe selective for PEK mRNA was used for in situ hybridization experiments. Autoradiographic analysis revealed that Raclopride slightly but significantly increased [^{125}I]NCQ-298 binding in the STR (10%), subthalamic nucleus (16%) and substantia nigra compacta (12%), whereas SCH-23390 significantly enhanced [^{125}I]SCH-23982 binding in the EP (43%), STR (17%) and substantia nigra reticulata (32%). Striatal PEK mRNA levels were significantly elevated by Raclopride (16%), SCH-23390 (41%) and by the combination of Raclopride and SCH-23390 (62%). Two-way ANOVA of the PEK mRNA data revealed that Raclopride and SCH-23390 enhance PEK mRNA expression through separate mechanisms. Supported by grant #8318 and #11274 from the Swedish MRC.

750.5

RAPID EFFECTS OF INTRASTRIATAL QUINOLINIC ACID INJECTIONS ON PALLIDAL ENKEPHALIN, SUBSTANCE P AND GAD-67 IMMUNOREACTIVITY IN RATS. Y. Bordelon* and M-F Chesselet
Department of Pharmacology, U. of Penn, Philadelphia, PA 19104.

Intrastriatal injections of quinolinic acid (QA), an NMDA receptor agonist, produce a loss of medium spiny projection neurons (which contain glutamic acid decarboxylase, and either enkephalin or substance P), and sparing of interneurons. Hence, they replicate the neurodegeneration found in Huntington's disease. We have previously shown that DNA strand breaks are present in striatal neurons in HD and after QA injections. DNA damage was detected 12, but not 10 hours after QA injections. Expression of met-enkephalin (ENK), substance P (SubP) and glutamic acid decarboxylase (Mr 67,000: GAD67), was examined with immunohistochemistry in striatal projection areas before and after DNA damage is evident in the striatum.

Male Sprague Dawley rats (250-300 gms) received a unilateral injection of QA (60 nmol in 0.5 µl) or saline over 5 min. in the striatum. This procedure induces a massive loss of striatal efferent neurons 2 weeks later (Qin et al. Exp. Neurol. 115, 1992,200-211). Rats were sacrificed 10 or 12 hrs after surgery by transcardial perfusion with 4% paraformaldehyde under deep anesthesia. Sections were processed for immunohistochemistry with avidin-biotin-peroxidase and diaminobenzidine-HCl as the chromogen. None of the rats sacrificed 10 hrs after QA injection in the striatum showed conspicuous changes in ENK immunoreactivity (IR) in the ipsilateral globus pallidus (GP). In contrast, a marked decrease in ENK IR was already observed in some of the rats sacrificed 12 hrs after QA injection. In these same animals GAD 67 IR in the GP and SP IR in the entopeduncular nucleus were also decreased. Thus, intrastriatal QA induces a rapid decrease in GAD and neuropeptide expression in axons of striatal efferent neurons, which appears to coincide with the onset of detectable DNA damage in their cell bodies. Supp. by MH-44894 and F-30-MH-10890

750.6

EXPRESSION OF ONCOGENE C-FOS PROTEIN IN THE MARGINAL DIVISION OF THE STRIATUM AND MEYNERT'S BASAL NUCLEUS FOLLOWING CHEMICAL INJURY OF THE RAT BRAIN. S.Y. Shu*, X. Bao and S. Li. Dept of Neurobiology, First Military Med. Univ., Zhu-Jiang Hospit. Guangzhou, 510282, China.

c-Fos induction can occur as a consequence of synaptic activation. This has led to the proposal that c-Fos immunohistochemistry might be used to map functional pathways in the CNS. In our previous study, a new subdivision, the marginal division (MrD), was found in the striatum and it projects to the area of Meynert's basal nucleus. The depression of the learning and memory behavior would be induced by injury of MrD. In the present study, the functional relationship of MrD and Meynert's basal nucleus was investigated by injection of Kainic acid (KA) in the MrD and Meynert's basal nucleus to induce c-Fos-like protein expression in the rat brain. 0.1-0.2% KA was injected in the brain with a stereotaxic instrument.
The rat was perfused 6-8 hours after injection. The brain was removed and sectioned. The sections were processed with c-Fos immunohistochemistry method. The results are: 1) Positive c-Fos protein expression was found in the amygdala and hippocampus after KA injecting in the Meynert's nucleus. The result showed that KA acid injury could induce c-Fos expression to show functional connections in the brain; 2) Positive c-Fos expression was observed in the MrD following KA injecting in the Meynert's nucleus; 3) Positive expression of c-Fos was found in the Meynert's nucleus by injection of KA in the MrD. The results indicate that there are functional connections between the MrD and the Meynert's basal nucleus. This relationship probably sponsors the learning and memory function of the MrD.(supported by NSFC 39270248)

750.7

VENTRAL MESENCEPHALIC NEURONS RETROGRADELY LABELED WITH WGA-HRPapoGOLD OR FLUORO-GOLD FOLLOWING INJECTIONS IN THE SHELL, CORE AND ROSTRAL POLE OF THE NUCLEUS ACCUMBENS: IMMUNOHISTOCHEMICAL AND MORPHOMETRIC ANALYSIS Y. Tan, J.S. Brog*, E.S. Williams and D.S. Zahm. Dept. of Anatomy and Neurobiology, Saint Louis University School of Medicine, St. Louis, Missouri 63104.

The mesotelencephalic projection comprises a morphologically and neurochemically heterogeneous population of axons. Ventral mesencephalic dopamine (DA) neurons exhibit several distinct types of somatodendritic morphology. Some studies have concluded that certain morphologically distinct dopaminergic cell types are associated with topographically and morphologically specialized axon projections. In the present study, either WGA-HRPapoGOLD or Fluoro-Gold (FG) were used to retrogradely label neurons in the ventral mesencephalon following injections that were relatively restricted to the core, shell or rostral pole of the nucleus accumbens. Retrogradely labeled neurons were evaluated for calbindin immunoreactivity, perikaryon diameter and labeled dendrite length. Following all injections except those in medial shell, calbindin D-28kD immunoreactivity was colocalized in a subset of retrogradely labeled neurons. Mean perikaryon diameter of FG labeled neurons was significantly less following injections in the medial shell than after injections in the core and rostral pole. Likewise, the median length of FG-labeled dendrites was significantly less following injections in the medial shell than following injections in the core and rostral pole. One hundred neurons from each of three cases for each subterritory were evaluated with a randomized ANOVA and post-hoc Tukey's protected T test. The data are consistent with a small to moderate component of midbrain DA/calbindin projections to core-rostral pole and a morphologically distinct population of DA neurons projecting to medial shell. NIH NS-23805 (NINDS).

750.8

FOCAL 6-HYDROXYDOPAMINE LESIONS IN NUCLEUS ACCUMBENS CORE AND SHELL ENHANCE FOS IMMUNOREACTIVITY PREFERENTIALLY IN TERMINAL REGIONS OF THE DOPAMINERGIC MESOTELENCEPHALIC PROJECTION. M.A. Chapman* and D.S. Zahm. Dept. of Anatomy and Neurobiology, Saint Louis University School of Medicine, St. Louis, Missouri 63104.

The nucleus accumbens (Acb) has been designated as a critical site of sensory-limbic-motor interface, neuroanatomically situated to mediate behaviors essential to the organism's survival. The Acb may be divided into subterritories based on neurochemical, morphological and hodological properties. Given the importance of this structure in theories of drug dependence and schizophrenia, it is critical to determine whether the different connectivity patterns of Acb subterritories are functionally relevant. In order to address this question, 6-hydroxydopamine microinjections were made in the Acb core or shell of male Sprague-Dawley rats. The lesion sizes and locations were precisely evaluated using tyrosine hydroxylase immunohistochemistry and Fos expression was assessed immunohistochemically at several post-lesion time points. Preliminary evaluation of the material revealed a robust enhancement of Fos immunoreactivity bilaterally in the medial prefrontal, insular and piriform cortices, septum, Acb and medial caudate-putamen of lesioned animals as compared to ascorbate vehicle and saline controls. Control animals exhibited moderate numbers of Fos-IR cells in the same brain regions; therefore, the numbers of Fos-positive cells will be quantified and statistically compared. Insofar as all of these areas receive mesotelencephalic dopamine input from ventral mesencephalic neurons within the projection field of the Acb, it is hypothesized that Fos immunoreactive neurons are disinhibited following focal Acb lesions through increased inhibition of midbrain dopamine neurons. Supported by USPHS NIH NS-23805 and NS-07254 (NINDS).

750.9

METHYLPHENIDATE TREATMENT AND GENE EXPRESSION IN THE VENTRAL TEGMENTAL AREA, SUBSTANTIA NIGRA, NUCLEUS ACCUMBENS AND STRIATUM IN RATS. M. Mercugliano*,H.Q. Nguyen, A. Cnaan. Children's Seashore House & Departments of Pediatrics and Biostatistics, University of Pennsylvania, Philadelphia, PA. 19104.

Methylphenidate (MPH) is a dopamine re-uptake blocker with effects on mesolimbic and mesostriatal dopamine (DA) systems. To identify neuronal subgroups involved in MPH effects, *in situ* hybridization histochemistry was performed after acute (at 7 wks) or chronic ip treatment (twice daily, 4-7 wks) with saline, low- (1.25 mg/kg) or high-dose (12.5 mg/kg) MPH. mRNA labelling for TH, GAD_{67} and DA_2 receptors were quantified at the single cell level in the VTA and SN, and GAD_{67}, DA_1 and DA_2 receptors and PPE regionally in the NA and STR. Locomotor activity, stereotypy and rearing were measured on days 1, 7, 14 and 21. After acute treatment, all behaviors were increased in the high-dose group ($p's \leq 0.005$) and there were no changes in mRNA labelling. With chronic treament, activity was highest in the high-dose group ($p = 0.0005$), and effects were largest on day 1 ($p's \leq 0.004$) . Stereotypy showed dose-dependent increases ($p < 0.00005$) without time effects. Rearing was increased in the high-dose group ($p = 0.0006$), with marginal effects on day 21. Chronic treatment with either dose resulted in increased TH labelling in the VTA and SNC ($p's \leq 0.05$). High-dose treatment resulted in increased labelling for PPE in the STR ($p < 0.01$). Mazindol binding was unchanged suggesting that this was not due to loss of DA terminals. Chronic MPH treatment alters gene expression in VTA and SNC DA neurons and STR efferent neurons which express PPE. Supported by Tourette Syndrome Association, HD28815 and HD26979.

750.10

THE DISTRIBUTION OF MU OPIOID RECEPTORS DEFINES THE CORE AND SHELL OF THE HUMAN NUCLEUS ACCUMBENS.

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Selective mu opioid receptor autoradiography with [^{3}H]DAMGO was used to examine regional and subregional mu receptor distribution patterns at five rostrocaudal levels through the human striatum. [^{3}H]DAMGO binding densities were measured in the individual striatal nuclei and in subregions. The distribution of [^{3}H]DAMGO binding sites has a strongly heterogeneous character. At the regional level a U-shaped distribution of density values was observed along the rostrocaudal axis, with highest values in the rostral- and caudalmost levels. Furthermore, a dorsal-to-ventral high-to-low gradient was found, with lowest binding densities in the ventral one-third of the Putamen (Put) and in nucleus accumbens (Acb). Binding in Caudate (Cd) and Put did not show a patch-matrix-like pattern as in the rodent. In the Acb, areas of low, intermediate and extremely high binding density were present. Comparison with ligand binding patterns of other receptors, e.g. the kappa opioid receptor labeled with [^{3}H]bremazocine, indicated that the low-density area represents the "core" region of the Acb, whereas the "shell" is characterized by intermediate density. Along the ventral periphery of the Acb and Put, a string of smaller regions were found that displayed the highest binding values of the entire striatum for [^{3}H]DAMGO. These regions could also be recognized in the distribution of the kappa opioid and dopamine D_1 receptors and in the cyto- and myeloarchitecture of the Acb. Because of these special features they are referred to by the acronym NUDAP: Neurochemically Unique Domains of the Accumbens and Putamen.

750.11

LOCALIZATION OF 5-HT2a RECEPTOR mRNA IN OPIOID NEURONS OF THE VENTRAL STRIATUM IN THE RAT. M.J. Mijnster, K. Koskuba, G.J. Docter, L.D. Loopuijt* and P. Voorn. Graduate School Neurosciences, Vrije Universiteit, Dept. of Anatomy, Amsterdam, the Netherlands.

The serotonin 5-HT2a receptor may be involved in the regulation of the opioid peptide dynorphin in the ventral striatum, as is suggested by changes in preprodynorphin (ppdyn) mRNA after a lesion of the serotonergic system. On the other hand, enkephalin synthesis does not seem to be affected by a serotonergic lesion as there is no change in preproenkephalin (ppenk) mRNA levels. We set out to investigate whether there is an anatomical basis for these different responses of opioid neurons. Double-labeling *in situ* hybridisation experiments were performed to demonstrate 5-HT2a and ppenk/ppdyn mRNA in the same sections. 5-HT2a mRNA was visualised with a ^{35}S-labeled riboprobe, whereas a digoxygenin-labelled riboprobe was used to demonstrate ppenk/ppdyn mRNA. Opioid neurons were identified and overlying grains generated by the radioactive probe were counted with an IBAS image analysis system. Our results demonstrate that 5-HT2a mRNA is localized in both enkephalinergic and dynorphinergic cells. In different subregions of the ventral striatum (medial shell, core, olfactory tubercle), 40-45% of the enkephalin neurons contain detectable levels of 5-HT2a mRNA. However, in the lateral shell the percentage of co-localisation is significantly higher (60%) than in the other subregions. Our results further indicate that 35-45% of the dynorphin cell population in the core and medial/lateral shell synthesizes the 5-HT2a receptor. In the olfactory tubercle, there is a significantly higher degree of co-localization (65%) compared to the other ventral striatal regions. On the basis of these data, we would suggest that 5-HT2a receptor manipulation can directly affect subpopulations of both types of opioid output-neurons. The magnitude of the effect of 5-HT2a receptor manipulation might differ between subregions of the ventral striatum.

750.12

CELLULAR LOCALIZATION OF D1 AND D2 DOPAMINE RECEPTOR mRNA IN THE ACCUMBAL-PALLIDAL AND ACCUMBAL-VENTRAL TEGMENTAL AREA PATHWAYS IN THE RAT X.-Y. Lu, L. Churchill and P. W. Kalivas* Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA.

The involvement of the ventral pallidum in mediating motor activity is thought to arise via afferent projections from the nucleus accumbens which in turn receives the dopaminergic input from mesencephalon. Two major families of dopamine receptors have been characterized in the central nervous system and designated D1 and D2 dopamine receptor subtypes. Using retrograde labeling methods combined with in situ hybridization, this study examined the postsynaptic distribution of accumbal D1 and D2 dopamine receptors following deposits of Fluoro-gold in the ventral pallidum (VP) and ventral tegmental area (VTA). The data indicated that the portion of retrogradely labeled neurons with Fluoro-Gold deposits in the VP that contained messenger RNA for D2 dopamine receptor was 19%-37% in the shell, 19%-30% in the core of nucleus accumbens, whereas 40%-67% and 50%-73% were double-labeled neurons for D2 receptor messenger RNA in the shell and core subcompartments, respectively. Preliminary evidence also showed double-labeled neurons with Fluoro-Gold deposits in VTA for D1 receptor messenger RNA ranged from 64% to 100% along a caudal-rostral gradient. These data demonstrate that both D1 and D2 receptors, predominantly D2 receptors, exist in the pathway from the nucleus accumbens to ventral pallidum, suggesting D1 and/or D2 receptors on accumbal neurons may directly modulate neurotransmitter release in the ventral pallidum, alter the firing frequency of pallidal neurons, and subsequently affect locomotor activity. This study also reveal that most accumbal neurons projecting to VTA contain D1 receptor messenger RNA. This suggests that dopamine release may act on D1 receptors in the nucleus accumbens to regulate the activity of dopamine cells in the VTA.

750.13

Lithium induces c-fos expression selectively in the "hippocampal district" of limbic striatum. **A. Jayaraman *, H. Kiba and M. Cola. Dept. of Neurology, LSU Sch. of Med. New Orleans, LA 70112.**

Lithium is the most effective agent for the treatment of manic depressive disorder. The forebrain dopaminergic system, among others, has been suggested to play a major role in acute manias. To identify the neuronal circuitry that is responsive to lithium, we mapped lithium induced Fos expression in the forebrain. Injections of lithium chloride induced Fos expression selectively in the "hippocampal district" of the accumbens, but not in rest of striatum. Numerous Fos positive neurons were noted in the hippocampal CA1, CA2 regions, cingulate, prelimbic, infralimbic, agranular insular and pyriform cortex, but only a few Fos positive cells were noted in deep layers of many cortical areas. Fos reactive cells were restricted to the midline and intralaminar nuclei of thalamus. Cells with Fos-Li were conspicuously absent in VTA & substantia nigra complex. The results suggest that the hippocampal district of accumbens & hippocampal and thalamic regions that converge on to this specific area of limbic striatum may mediate lithium induced benefits on acute & recurrent manic attacks. **Supported by Smokeless Tobacco Research Council, NY & Department of Defense.**

750.14

TO WHAT EXTENT IS THE BASAL GANGLIA ORGANIZATION OF ANURAN AMPHIBIANS COMPARABLE TO THAT OF AMNIOTES? W.J.A.J. Smeets*[1], O. Marín[2] and A. González[2]. [1]Graduate Sch. Neurosci., Dept. Anatomy & Embryology, Vrije Univ., Amsterdam, The Netherlands. [2]Dept. Cell Biology, Univ. Complutense, Madrid, Spain.

Recent studies have revealed that there are many similarities in the organization of the basal ganglia (BG) between reptiles, birds, and mammals. The intriguing question raises, therefore, whether this basic organization exists also in anamniotes. In amphibians, not only a dopaminergic (DA) cell group in the midbrain, but also distinct plexuses of DA fibers in the ventromedial (accumbens) and ventrolateral (striatum) wall of the telencephalic hemisphere have been identified. In contrast to amniotes, the midbrain DA cell group of amphibians is rostral continuous with DA cells in the dorsomedial part of the posterior tubercle (TPm), and does not extend caudal to the oculomotor nerve. It is also impossible to recognize separate A8-A10 cell groups, indicating a putatively different organization of BG in amphibians. To get more insight into the organization of the DA mesotelencephalic connections, combined tract-tracing/immunohistochemical techniques have been used. Retrograde tracers (biotinylated dextran amine or Texas-Red conjugated dextran amine) were applied to the basal forebrain of anurans (*Rana perezi*, *Xenopus laevis*). As marker for DA cells, we have used tyrosine hydroxylase (TH) immunohistochemistry. Experiments involving the accumbens yielded some double labeled cells in the TPm and, more frequently, in the midbrain tegmentum. Involvement of the striatum, on the contrary, resulted in many double labeled cells in the TPm at intermediate levels, but not in the midbrain. Thus, the nucleus accumbens and the striatum of anurans receive topographically organized DA inputs which, however, display a preferential caudal-to-rostral, instead of a medial-to-lateral arrangement as demonstrated for amniotes. Supported by DGICYT PB93-0083

750.15

ASTROCYTIC GLUTAMATE AND DOPAMINE RECEPTOR SUBTYPE CO-LOCALIZATION IN THE RAT SUBSTANTIA NIGRA. A.Mateescu-Cantuniari, J.W. Patrickson, K.H. Thomas, J.A. Whittaker.*
Department of Anatomy, Morehouse School of Medicine,Atlanta,GA,30310.

Abnormalities in CNS dopaminergic neurotransmission has been hypothesized to be related to possible excitotoxic actions of excitatory amino acids (EAA). There is increasing evidence that astrocytes may have a functional role in EAA-mediated brain injury and, as well, may mediate their phenotypic expression. Electrophysiological studies have indicated direct signaling between astrocytes and neurons suggesting the possibility of an astrocytic involvement in nigral degeneration and related disease etiology.[1] We have previously localized glutamate and dopamine receptor subtypes within the rat substantia nigra (SNc). This study was designed to further investigate these receptor subtype distributions and their relationships to astroglia within the pars compacta of the rat SNc. Using immunolabelling techniques, we studied D2/3, GluR2/3, NMDAR1, and NMDAR 2A/B receptor subtype expressions, in the adult rat brain. Commercially available primary antibodies (Chemicon; Sigma) directed against the respective receptor subunits and against the astrocytic marker, GFAP, were used in this study. FITC labeled GluR2/3, D2/3, NMDAR1 and NMDAR 2A/B receptor immunoreactivity was observed in a heterogenous pattern throughout the SNc in close association with GFAP/Rhodamine labeled astrocytes. Our results suggest DA and glutamate receptor subtypes are co-localized in the cytoplasmic compartment of SNc astrocytes.
SUPPORT: NIH grants S06GM08248 and 3G12RR03034.

[1]M. Nedergaard, Science, 263: 1768-1771, 1994.

750.16

APOMORPHINE TRANSIENTLY REDUCES SUBSTANCE P-LIKE IMMUNOREACTIVITY IN THE SUBSTANTIA NIGRA IN 6-OHDA RATS. S Gancher*, A Mayer, S Youngman. Department of Neurology, Oregon Health Science University, Portland, Oregon, 97222.

Substance P is a peptide which is contained in striatonigral neurons and present in high amounts in the SN. Lesioning and repeated drug treatment affect nigral substance P content; 6-OHDA lesioning reduces nigral substance P levels and daily L-DOPA treatment reverses this decrease. In the current study, we wished to determine if acute drug treatment also differentially affects substance P content in the lesioned SN. Eight rats with 6-OHDA lesions received 8 daily doses of apomorphine (0.1 mg/kg), a regimen which produced behavioral sensitization. The next day, four 3.2 mg/kg apomorphine doses were administered at 2 h intervals, a regimen which produced tolerance. Four rats were perfused 1-2 h later; the other four were perfused 1 week later. The density of substance P-like immunoreactivity (SPLI) in the SN on the 6-OHDA and normal side were compared by image analysis. In the acutely treated rats, the SPLI in the lesioned SN was reduced relative to the other side, whereas the rats untreated for 1 week after drug treatment exhibited symmetric staining. These findings suggest that administration of a dopamine agonist temporarily reduces substance P levels in the SN ipsilateral to a 6-OHDA lesion, possibly reflecting an increase in striatonigral neurotransmission from the dopamine-depleted striatum. The observation that acute and chronic treatment produce different behavioral effects and corresponding changes in nigral substance P levels suggests that substance P may have a modulatory role in affecting dopaminergic response in animals with nigrostriatal lesions.

750.17

FUNCTIONALLY AND ANATOMICALLY DISTINCT DOPAMINE SYSTEMS IN THE MEDIAL PREFRONTAL CORTEX: AN APPRAISAL. A. Y. Deutch,* Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT and Veterans Administration Medical Center, West Haven, CT 06516.

Several cortical regions in the rat receive dopamine (DA) innervations, the most prominent of these being the medial prefrontal cortex (PFC). DA projections to the PFC are derived from cells in the ventral tegmental area (VTA). The mesoprefrontal cortical DA innervation has been considered to be a functionally homogeneous DA innervation. However, the DA innervation of the rodent PFC appears to be homologous to several spatially distinct in the primate that have different functional attributes. We have recently observed that several DA innervations of the PFC in the rat can be anatomically, biochemically, and molecularly distinguished, and that these different DA innervations respond different to different challenges, including mild stressors and antipsychotic drugs.

We propose that there are several different DA innervations of the PFC in the rat that follow in part cytoarchitectonically-defined regions, that these different DA innervations of PFC regions are derived DA neurons in different subnuclei of the VTA, and that various challenges activate both a DA innervation of a particular part of the PFC (e.g., the infralimbic or dorsal prelimbic cortex) and concomitantly a particular cluster of midbrain DA neurons. Supported in part by MH-45124, the NPF Center at Yale, and the VA Schizophrenia and PTSD Centers.

CEREBELLUM: PHYSIOLOGY

751.1

THE CEREBELLAR GAIN CHANGE HYPOTHESIS EVALUATED IN THE ANESTHETIZED RABBIT P.L. DiGiorgi, C.I. De Zeeuw, S.K.E. Koekkoek, and J.I. Simpson*. Dept. Physiology and Neuroscience, NYU Med. Ctr., NY, NY 10016

The gain change hypothesis proposed by Ebner and Bloedel states that the climbing fiber input to a Purkinje cell (P-cell) produces a short-lasting enhancement of its responsiveness to mossy fiber inputs. We examined the simple spike (SS) activity patterns that followed the complex spike (CS) of floccular P-cells in ketamine-xylazine-acepromazine anesthetized rabbits. We recorded from 43 P-cells whose CSs were modulated by optokinetic stimulation (OKS). Three conditions were investigated: spontaneous activity in light and dark and constant speed OKS. Data analysis included CS-SS cross-correlation and SS auto-correlation. P-cells were assigned to one of three categories: pure pause (PP), pause-facilitation (PF), or pause-reduction (PR). To be categorized as a PF (or a PR) cell, the average SS activity during at least one 20 msec period within the first 50 msec after the end of the CS pause had to be significantly ($P<0.01$) greater (or less) than the average SS activity during the 100 msec prior to the CS. Of the 43 P-cells, 26 (60%) were PF cells, 9 (21%) were PR cells, and 8 (19%) were PP cells. The categorization distribution was condition independent (Fisher exact test). For about 80% of the cells, the SS auto-correlograms showed a pronounced, exponentially damped oscillation (range, 25-100Hz), also condition independent. In comparison to findings in the awake rabbit (Simpson, Wylie and De Zeeuw, submitted), the percentage of P-cells showing enhancement of responsiveness to mossy fiber inputs doubled and the SS rhythmicity markedly increased. Thus, ketamine-xylazine-acepromazine anesthesia promotes enhancement and SS rhythmicity. (Supported by NS-13742).

751.2

ANALYSIS OF CA^{2+} SPIKE AUTORHYTHMICITY IN CEREBELLAR PURKINJE CELLS. Y. Etzion * and Y. Grossman. Department of Physiology, Faculty of health Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel.

Spontaneous Ca^{2+} spike activity in cerebellar Purkinje cells is characterized by a regular alternation between bursting and quiescence periods and a propensity for firing in doublets. We studied the underlying mechanisms of this behavior in guinea pig cerebellar slices. Slices were constantly perfused with 5mM KCl Ringer's solution equilibrated with 95% O_2, 5% CO_2 at 30°C. Spontaneous Ca^{2+} spike currents were recorded by using a macropatch clamp technique before and after blocking sodium currents with 0.5μM TTX. TTX significantly increased active period duration to 149.5±44.9% (mean ±SD, n=14), silent period duration to 142.1±53.8% and the mean inter spike interval (ISI) to 150.9±63.4% but did not change doublet spiking. Application of CNQX (10μM) and Bicuculline (20μM) did not mimic TTX effects. Heating to 33°C enhanced doublet firing, while cooling to 28°C markedly increased mean ISI and almost abolished doublet spiking. Increasing $[K^+]_o$ to 7.5mM, addition of 4-AP (1μM), or TEA (0.1mM) greatly increased doublet spiking and could counteract the effect of cooling. Whereas, reducing $[K^+]_o$ to 3mM, like cooling, almost abolished doublet spiking. The ISI within doublets changed gradually by these manipulations from few ms to approximately 40 ms. These results indicate : a) Invasion of somatic Na^+ spikes may affect dendritic Ca^{2+} spike autorhythmicity. b) Existence of a highly 4-AP sensitive K^+ current which reduces doublet spiking. c) The range of modulation of ISI within doublets can not be attributed to spike propagation between two zones of the dendritic tree, and therefore support the notion of a single site for doublet generation.

751.3

Oscillatory activity at 14-16 Hz in the cerebellar cortex. Relationship with movement initiation in the behaving monkey. J-P Pellerin,C.Valiquette,M-T.Parent,Y.Lamarre*. Centre de recherche en sciences neurologiques, Université de Montréal.

A monkey was trained to move the arm about 1 sec. following an auditory stimulus. In the paramedian lobule of the cerebellum we recorded rhythmic bursting of thin, small spikes at the frequency of 14-16 Hz. The amplitude of local field potential oscillations at the same frequency was maximal in the granular layer and Purkinje cell activity recorded in adjacent layers was not modulated at that frequency. So far we have been able to establish the following properties of this oscillatory process:**1.** It is very much decreased during reduced state of vigilance and after administration of harmaline;**2.** it is reduced and disorganized some 150-250 ms after the external cue, resumes 200-250 ms later and stops abruptly at about movement onset;**3.** the oscillations are enhanced in the 400-500 ms period preceding movement onset. This is not observed with spontaneous movement;**4.** olivo-cerebellar activity tends to increase 150-250 ms after the external cue at a time where the rythmic process appears to be reset. More investigations are needed to establish the functional role of this oscillatory process which could be involved in the precise timing of movement onset in response to external events. Supported by MRC of Canada.

751.4

CEREBELLAR GRANULE CELL RESPONSES IN THE AWAKE, FREELY-MOVING RAT: THE IMPORTANCE OF TACTILE INPUT. M.J. Hartmann* and J.M. Bower, Division of Biology, Caltech, Pasadena, CA, 91125.

Using a light-weight microdrive developed in our lab, we have recorded activity of the cerebellar granule cell layer (GCL) from multiple sites in Crus IIa in awake, freely-moving rats. We have previously shown that in the anesthetized animal, cells in this region of the cerebellum respond to light touch of perioral structures. During chronic electrode implantation we determined the receptive field at each recording site, and we later confirmed these fields in the awake rat. Behaviors were videotaped and synchronized with the recorded neural signals.

We compared the spatio-temporal patterns of GCL activation during eating, grooming, drinking, and exploratory behaviors. Each behavior elicited unique and repeatable patterns of neural activity. For the observed behaviors, activity recorded on each electrode was largest when the rat's own movements resulted in tactile stimulation of the appropriate perioral receptive field. In contrast, activity remained close to background levels during perioral behaviors that did not directly stimulate the receptive field. For example, substantial GCL activity was observed when the rat palpated food with its lips, while chewing motions elicited little or no response.

During the highly rhythmic and repeatable sequences of rat drinking, each lick elicited two distinct bursts of granule cell activity. Analysis of the video-tape data suggested that these bursts were related to tongue protrusion and retraction past the perioral receptive fields. During some drinking sequences, rats received an air-puff stimulus to perioral regions; when the stimulus was directed towards a receptive field, large responses superimposed on the rhythmic activity associated with licking. We conclude that GCL responses in crus IIa of behaving rats seem to be most directly related to tactile stimulation of perioral surfaces.

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751.5

FUNCTIONAL ORGANIZATION OF THE RAT CEREBELLAR CORTEX ELUCIDATED BY PRINCIPAL COMPONENT ANALYSIS OF OPTICAL SIGNALS C. Hanson, Q.-G Fu*, G. Chen, T.J. Ebner Departments of Neurosurgery and Physiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455

The highly regular cellular lattice and circuitry of the cerebellar cortex and its afferent and efferent systems are organized around several spatial plans. Optical imaging is one approach used to record the patterns of activity in the cerebellar cortex. In this study, we used principal component analysis (PCA) to extract spatial patterns of activity from optical images of rat cerebellar cortex under conditions of peripheral or surface stimulation.

Optical signals from stimulus evoked fluorescence changes in cerebellar cortical area Crus II were recorded with a CCD camera in anesthetized rats (ketamine / xylazine). The cortex was stained by superfusion (2.5mg/ml) and intravenous injection (10mg/ml) of the pH sensitive dye neutral red. Stimuli consisted of direct cortical stimulation to evoke responses from parallel fibers or bipolar electrical stimulation of the face.

Principal component analysis of the images obtained with direct surface stimulation showed a clear medial-lateral band of activity consistent with activation of a band of parallel fibers. The PCA analysis extracted parasagittal bands in Crus II from stimulation of the ipsilateral face. In both cases these patterns were derived from the first few principal components (ie. those accounting for a large majority of the variance). These patterns are consistent with those obtained from simple frame averaging then subtraction of control images from stimulation images. The results also suggest that principal component analysis provides the additional power to extract other features of the image, such as the vasculature. This approach offers the potential to not only separate out the various contributions to the total fluorescence change but to quantitize them precisely. Supported by NIH Grants PO1 NS31318 and T32 GM08471

751.6

OPTICAL IMAGING OF THE PARASAGITTAL ORGANIZATION IN THE RAT CEREBELLAR CORTEX *IN VIVO* USING THE PH SENSITIVE DYE NEUTRAL RED. G. Chen, C. Hanson, T.J. Ebner*. Departments of Neurosurgery and Physiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455.

A prominent organizational feature of the cerebellar cortex is that some of its afferent and efferent projections are organized into parasagittal zones. In this study we used optical imaging techniques to demonstrate that stimulation of the face activates parasagittal zones in the rat cerebellar cortex *in vivo*.

Crus II was exposed in anesthetized rats (ketamine/xylazine) and stained with the pH sensitive dye, neutral red (2.5mg/ml), by superfusion for 2-3 hours. Staining was supplemented by intravenous administration of 1ml (10mg/ml) neutral red delivered over one hour. After washout, Crus II was imaged (100ms exposure, excitation at 540nm, emission>620nm) with a cooled CCD camera. A series of images was acquired before, during, and after ipsilateral bipolar stimulation of the face (500μs, 5Hz, 10-20s). Subtraction of prestimulation control images from each image obtained after stimulation yielded a spatial and temporal map of the pattern of activation in the cerebellar cortex. The most striking feature of the optical responses was the presence of 3-5 parasagittal zones of increased fluorescence. These zones spanned both Crus IIa and IIb and were 100-500μ in width. The optical response was abolished by TTX and reduced by CNQX. The time course of the optical response peaked after stimulation ended and had a duration of 30 to 120 seconds. The size of the optical signals were as large as 3-4% change in background fluorescence, and the signals were obtainable for up to four hours after staining. Previous work suggests that these optical signals reflect shifts in intracellular pH due to neuronal activity.

These observations are the first optical demonstration of the activation of parasagittal zones in the cerebellar cortex in response to peripheral stimuli. Supported by NIH Grants PO1 NS31318 and T32 GM08471.

751.7

RELATIONS BETWEEN PURKINJE CELL SIMPLE SPIKE ACTIVITY AND MOVEMENT VELOCITY AND POSITION IN MONKEYS USING A NOVEL, TWO-DIMENSIONAL TRACKING TASK. J. D. Coltz* and T. J. Ebner, Graduate Program in Neuroscience and Depts. of Neurosurgery and Physiology, University of Minnesota, Minneapolis, MN 55455.

Previous electrophysiological studies of cerebellar Purkinje cells in our laboratory (Fu et al., 1994) found a significant correlation of simple spike discharge with the movement parameters direction, distance, and target position. However, the variance in cell discharge accounted for by these parameters was moderate, suggesting that other variables are needed to describe more fully the activity of these cells. The present study employed a novel, two-dimensional visuomotor task that required monkeys to track moving targets at 5 different velocities, reaching from a centrally located start position to endpoints in 8 different directions. The velocity profiles of these moving targets were bell-shaped, and the peak velocities ranged from 2 to 6 cm/sec. Single trial analyses of the Purkinje cell simple spike discharge utilized a multiple regression model in which the temporal profile of the cell discharge was fitted to the profiles for hand position, velocity, and acceleration. The overall R^2 for all cells was greater than that obtained in the earlier study, having a mean ± s.d. of 0.42 ± 0.12. The relative contributions of each of the parameters were quantified by calculating partial R^2 values. Velocity information contributed more to the overall strength of the model than did position or acceleration information in 19 of 30 cells. In 11 of 30 cells, position information contributed more than that about velocity or acceleration. These preliminary findings indicate that movement velocity and position are important correlates of simple spike activity in the primate cerebellum. Supported by NIH grants NS 18338 and NS 31530.

751.8

ESTRADIOL FACILITATES SYNCHRONIZED OLIVARY OSCILLATIONS DURING WHISKER MOVEMENT IN THE RAT INDEPENDENT OF INPUT FROM THE PRINCIPAL TRIGEMINAL NUCLEUS (PrV) OR THE SPINAL NUCLEUS OF V (SpV). R.L. Markowitz, J.K. Chapin, T.M. Fisher and S.S. Smith*Dept. of Anatomy and Neurobiology, MCP/Hahnemann Univ., Phila., PA.

Ongoing studies from this lab have demonstrated that 17β-estradiol (E_2), administered systemically at physiological doses or locally via an indwelling cannula, enhances synchronized, rhythmic discharge of neurons within the rostral dorsal accessory olive (rDAO) during rapid movement of the limbs. This effect is consistent with reports suggesting that increases in circulating levels of E_2 facilitate rapid, alternating movement of the limbs, and is also consistent with the reported functional role of olivary oscillations, as providing the frequency template for rapid limb movement (Llinas, 1991). For the present study, neurons from the whisker receptive area of the rDAO were recorded simultaneously with cells from corresponding areas of the PrV and SpV using chronically implanted microwire bundles (NB Labs, Denison, TX). Activity from these three CNS sites could then be monitored (Spectrum Scientific, Dallas) during active whisking 24 h following injection of E_2 (2 μgs, i.p. for 2 days). Results suggest that periods of active whisking were accompanied by rhythmic rDAO discharge at roughly the same frequency as the average frequency of the movement (~7-9 Hz). In contrast, the PrV and SpV demonstrated minimal oscillatory activity during whisking behavior. E_2 enhanced the oscillatory discharge of the rDAO both by slightly increasing the number of synchronized, oscillating cells (by ~20%) and by enhancing the amplitude of the oscillation (by ~100%). This effect was unaccompanied by facilitation of oscillatory activity in either the PrV or the SpV (15-16 cells recorded per CNS area). Preliminary videoanalysis of concurrent whisking activity, filmed during the recording paradigm, suggests that the rhythmic neuronal activity enhanced by E_2 was correlated with highly rhythmic whisking; E_2 administration produced whisking at a consistent, invariant frequency of 8.5 Hz compared to a highly variable whisking frequency observed under control, diestrous conditions (5-10 Hz). These results suggest that the facilitatory effect of E_2 on rhythmic, synchronized rDAO activity may underlie, at least in part, its observed behavioral effect in facilitating rhythmic whisker movement. (Supported by USAF 95-3-0136 to SSS and ONR N00014-95-1-0246, NS23722 to JKC.)

751.9

CODING OF DIRECTION AND POSITION BY DSCT NEURONS.
G. Bosco, R. Anafi and R. Poppele*. Dept of Physiology, Univ. of Minnesota, Minneapolis, MN 55455

Recent data showed that dorsal spinocerebellar (DSCT) activity may encode the direction of hindfoot displacements as well as relative foot position (Bosco & Poppele, J. Neurophysiol. 70:863,1993). It is not clear, though, if these two signals are temporally segregated or if they overlap in time.

We characterized the time course of DSCT directional tuning using a typical center-out paradigm, passively displacing the cat's hindfoot 2 cm in eight different directions in the sagittal plane. Unit activity recorded in each position was significantly modulated by movement direction for 5-6s following displacement, reflecting a broad directional cosine tuning.

In another experiment, the cat's foot was placed in several positions throughout the sagittal workspace, each position being approached from 3-4 directions in separate trials. For 60% of the cells, mean firing rates over 8s were significantly correlated with the position of the foot relative to the hip. However, only 30% of the cells showed significant modulation with position for the first 2s following displacement. The residual modulation was correlated with movement direction according to a directional cosine model. When this modulation component was subtracted from the total modulation over 8s, the overall correlation of activity with foot position improved and became significant in 75% of the cells.

The results suggest DSCT activity represents a combination of two concurrent signals: one related to the current foot position and the other to the direction of prior movement. The latter persists for several seconds after the movement.

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VESTIBULAR SYSTEM: VESTIBULAR NUCLEI

752.1

DEVELOPMENTAL CHANGES IN DISCHARGE PROPERTIES OF CHICK VESTIBULAR SENSORY NEURONS.
K.D. Peusner* and C. Giaume. Dept. Anatomy and Neuroscience Program, George Washington Univ., Washington, D.C. 20037.

The chick tangential nucleus is a vestibular nucleus in the brain whose principal cells (PCs) undergo major developmental changes in structure and membrane properties at critical embryonic and hatching ages. During that period, there is evidence to support that changes in membrane excitability are correlated with the outgrowth of dendrites, which start growing at 13 embryonic days (E13), and then double in length during the week between E15 and 1-2 days after hatching (N.Y. Acad. Sci. Abst., 1995). Using intracellular recording in brain slices, we have focused on the membrane responses of PCs to injected depolarizing current pulses of 400 msec duration with intensities ranging from 0.5 to 1.0 nA. At E13, all of the investigated cells (n = 4) responded with a single spike to 1.0 nA of depolarizing current. At E15-16, similar currents produced either a single spike (n = 3) or in 6 other cells, the depolarization induced the firing of several action potentials (APs). This firing was irregular in number, amplitude and time interval between APs and occurred with an average discharge rate of 32 APs/sec. At H1-2, PCs responded to the same depolarizing current by repetitive firing (discharge rate, 68 APs/sec) of APs with regularly-spaced intervals between the APs (n = 7). These observations indicate that by hatching PCs can respond to rapidly occurring synaptic events. Accordingly, parallel to the extensive outgrowth of dendrites in newborns, which could subserve an increasing number of synaptic inputs, PCs begin to acquire membrane properties which allow the cells to follow vestibular stimuli. Supported by NIH grant RO1-DC00970.

752.2

NEURAL CORRELATES OF VISUAL POSTURAL REFLEXES AND NEGATIVE PHOTOTAXIS IN LAMPREY.
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Eye illumination in lamprey evokes both negative phototaxis (NP), and a roll tilt towards the light (dorsal light response - DLR). DLR can outlast the stimulus with 30-60s, and reflects a change in the set-point of the vestibular roll control system. The neuronal correlate of DLR was investigated with *in vitro*-recordings from reticulospinal neurons (RSNs), which form the main descending system for roll control in lamprey. Optic stimulation gave a long-lasting potentiation of the vestibular response in ipsilateral RSNs, which indicates that the roll control system was reconfigured to stabilize a tilted orientation. The potentiation was largely due to increased excitability in pre-reticular interneurons. To elucidate which interneuronal pathways mediate DLR and NP, behavioural experiments were performed on cronically lesioned animals. Optic nerve fibres in lamprey project bilaterally to tectum, pretectum and thalamus ("lateral geniculate nucleus", LGN). These areas project further bilaterally to RSNs; commissural fibres first course ventrally and cross the midline in the basal plate. Ablation of the tectum, *potentiated* both DLR and NP: after illumination of the eye, the animal rolled several times around its longitudinal axis, and performed repeated yaw turns away from light. This could be explained by a general disinhibition of reticular cells after tectectomy. The same picture was seen after transection of the mesencephalic ventral commissure, which indicates that the tecto-reticular inhibition to a large extent is through crossing fibres. After unilateral ablation of the pretectum, which presumably also may damage some LGN cells and axons, both DLR and NP could still be evoked from the ipsilateral eye; contralateral visual responses were as a rule absent. Transection of the posterior commissure, which connects left and right pretectum, did not influence NP, nor DLR. Transection of the caudal diencephalic ventral commissure saved DLR, whereas NP was replaced with *positive* phototaxis. Cells in contralateral pretectum or LGN thus appear to play a major role both for NP and DLR, with fibres responsible for NP presumably crossing the midline at a diencephalic level.

752.3

VESTIBULAR CONTROL OF HEAD MOVEMENT IN SQUIRREL MONKEY: MORPHOLOGY OF INDIVIDUAL LATERAL VESTIBULOSPINAL AXONS.
R. Boyle*, D. Petrovic and J. Xu. Depts. Otolaryngology/Head-Neck Surgery & Physiology, Oregon Health Sciences Univ., Portland, OR 97201.

The lateral vestibulospinal tract (LVST) provides a pathway through which the vestibular-nerve afferents can affect head stabilization and movement. Individual axons of LVST neurons were intracellularly recorded to determine their short-latency input from the labyrinth and labeled using biocytin. Main morphological features examined are: axon trajectory in brainstem and cervical spinal cord, and termination pattern and segmental distribution in the cervical spinal cord; in several cases the axon's cell body was recovered in the vestibular nuclei. Antidromically identified lumbar-projecting LVST axons often travel in the ventrolateral funiculus, but can course in the lateral funiculus along the cervical cord. <10% of these axons provide a collateral input to the upper cervical ventral horn; the few axons that could be followed more posteriorly passed the cervicothoracic junction without branching. Lumbar-projecting LVST axons thus appear to provide a direct channel to the hindlimbs. LVST axons terminating in the upper cervical segments often enter the spinal cord in the ventral funiculus, but can course from the lateral to the ventral funiculi. The termination pattern of these axons depends on the axon's funicular location; extensive synaptic input is provided to ventromedial lamina IX, and laminas VIII and VII, thus targeting principally the motoneuronal cell groups. In some cases an axon travels and terminates like other LVST axons, but then changes funicular pathway and branches in a manner more characteristic of medial vestibulospinal tract cells. (Supported by PHS NINDS NS27050)

752.4

THE COERULEO-VESTIBULAR PATHWAY IN RATS, RABBITS, AND MONKEYS: QUANTITATIVE IMMUNOHISTOCHEMISTRY, NEUROTOXIN TREATMENT AND RETROGRADE TRACING.
R.J. Schuerger*, C.D. Balaban. Depts. Neurobiology and Otolaryngology, U. Pittsburgh. Pittsburgh, PA 15261

We examined the noradrenergic pathway from locus coeruleus (LC) to the vestibular nuclei (VN) in rats, rabbits, and monkeys. Noradrenergic projections were localized immunohistochemically with antibodies raised against catecholamine biosynthetic enzymes. The density of noradrenergic innervation was quantified. This analysis revealed a differential distribution of noradrenergic projections throughout VN. In all three species, innervation is highest in the lateral (LVN) and superior (SVN) VN. In rats, Deiters' neurons in LVN receive the densest projections. In monkeys, highest levels are seen in lateral portions of LVN, SVN, medio-rostral portions of prepositus hypoglossi and MVN, and to a lesser extent in group y. In rabbit, the highest innervation density was observed in SVN and LVN. Since immunoreactive fibers could be traced from LC to VN, the hypothesis that LC provides noradrenergic innervation of VN was tested by two methods. First, the neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4), was used to selectively destroy LC projections in rats. DSP-4 treatment greatly reduced noradrenergic innervation of VN. Second, retrograde tracing studies in the VN of rats (FluoroGold, rhodamine-dextran) and rabbits (HRP) indicated that the pathway originates only from the ipsilateral, ventral portion of caudal LC, subcoeruleus and A4. These results suggest that LC activity may play a role in modulating vestibular reflexes by direct action in VN and may therefor offer an anatomical substrate for increased vestibular performance during states of heightened arousal.

752.5

INDUCTION OF IMMEDIATE EARLY GENES DURING VESTIBULAR COMPENSATION. C.L.Darlington*, P.F.Smith, M.Dragunow" Dept. of Psychology, Univ. Otago, Dunedin, N.Z./"Dept. of Pharmacology, Univ. Auckland Med. School, Auckland, N.Z.

Vestibular compensation is aprocess of CNS plasticity that occurs following deafferentation of the vestibular nerve.Despite the severe ocular motor and postural symptoms that develop immediately following the lesion, many of these symptoms have disappeared within 2-3 days; this compensation process is correlated with a return of resting activity to type I neurons in the medial vestibular nucleus (MVN) ipsilateral to the lesion. Using specific antibodies for the immediate early gene (IEG) proteins, c-fos, c-jun and Krox 24, we analysed IEG induction in guinea pig MVN in 4 conditions: sham; immediately post unilateral surgicallabyrinthectomy (UL);10hs post-UL; 50 hs post-UL (n=3 each).c-fos was not induced in any condition; c-jun and Krox 24 were induced in the contralateral MVN at 10 hs post-UL only.It may be that c-fos is induced at another time post-UL, e.g 24 hs. The induction of c-jun is sometimes associated with cell death, however analysis of the contralateral MVN at 10 and 50 hs post-UL indicated no signs of apoptosis. At present, the significance of Krox 24 induction in the contralateral MVN is unclear.

752.6

EFFECTS OF ELECTRICAL STIMULATION ON VOR AND C-FOS LIKE PROTEIN EXPRESSION OF THE MEDIAL VESTIBULAR NUCLEI FOLLOWING UNILATERAL LABYRINTHECTOMY IN RATS. B.R. PARK*, M.S. KIM, M.Y. LEE, O.J. KIM. *Department of Physiology, Wonkwang University School of Medicine & Medicinal Resources Research Center, Iri 570-749, Korea*

The effects of electrical stimulation on vestibuloocular reflex(VOR) and c-fos like protein expression of the medial vestibular nuclei were investigated for 72 hours following unilateral labyrinthectomy(ULX) in Sprague - Dawley rats. Electrical stimulation with 1.0 ms and 100 Hz was applied to the lesioned vestibular system for 6 hours per day.

ULX caused spontaneous nystagmus with fast phase to the intact side, whose frequency was 3.9+0.2 beats/sec (M+SE) just after ULX but disappeared by postoperative 48 hours. On sinusoidal rotation of the whole body, eye movement with fast phase to the intact side persisted by rotation toward intact or lesioned side, but the eye movement induced by rotation toward the lesioned side recovered normal pattern by 24 hours at 0.1 Hz rotation, by 6 hours at 0.2 Hz. Directional preponderance which represents the symmetry of bilateral vestibular functions showed less than 20% on 48 hours, which implies the recovery of symmetry in bilateral vestibular functions. In electrical stimulation group, spontaneous nystagmus disappeared by 24 hours, and directional preponderance of VOR was less than 20 % by 24 hours. On immunohistochemistry of the medial vestibular nuclei the number of c-fos like protein expression was 5.0–8.0 in each nucleus before ULX, but the number increased to 81.5+6.3 in the lesioned side and 208.4+22.0 in the intact side on 2 hours. On postoperative 24 hours the number in the lesioned side was 55.8+1.8 and in the intact side was 7.0+3.0. However, 48 hours after ULX c-fos like protein was expressed equally in both nuclei. By electrical stimulation the number of c-fos like protein expression was symmetrical in both nuclei 24 hours after ULX. These results suggest that electrical stimulation ameliorates the recovery of VOR following ULX by modulation of the bilateral vestibular nuclear activity. Supported by MRRC sponsored by KOSEF and KOSEF(951-0708-021-2).

752.7

SYNAPTIC CONNECTIONS OF THE VESTIBULAR COMMISSURAL PATHWAY IN THE CAT. F.A. Chen* and R.F. Spencer. Department of Anatomy, Medical College of Virginia, Richmond, Virginia 23298-0709.

Vestibular commissural connections are believed to be mainly inhibitory in nature and are thought to play a role in the vestibulo-ocular reflex, the enhancement of vestibular responses, and recovery of function from peripheral lesions of the VIIIth nerve. In the present study, injections of biocytin were made into the medial vestibular nucleus of adult cats to anterogradely label commissural synaptic endings in the contralateral vestibular complex. By light microscopy, biocytin-labelled commissural axons were observed coursing medially from the injection site, crossing the midline ventral to the MLF, and forming terminal arborizations in homotopic regions of the contralateral medial vestibular nucleus. By electron microscopy, labelled commissural synaptic endings contained numerous small mitochondria and were classified on the basis of their content of spheroidal (64.1%), pleiomorphic (31.2%) or flattened (4.8%) synaptic vesicles. Cross-sectional areas of vestibular commissural endings ranged from 0.30 to 4.80 μm^2, with a mean of 1.37 ± 0.79 μm^2. Vestibular commissural synaptic endings were associated predominantly with distal dendrites, but also were seen in association with proximal dendrites and less frequently with somata and spine-like appendages. Postsynaptic diameters ranged from 0.31 to 9.34 μm^2 with a mean of 3.36 ± 1.96 μm^2. Quantitative analysis of the soma-dendritic distribution revealed that 81.3% of endings terminated on distal dendrites, while 12.6% and 5.1% of endings terminated on proximal dendrites and somata, respectively. Only 1.1% of endings were found to terminate on spine-like processes. In most instances, each vestibular commissural synaptic ending established synaptic contact with only one postsynaptic profile. However, multiple synaptic endings arose from single commissural axons and contacted the same postsynaptic process. The results of this study suggest that the vestibular commissural pathway in the cat, like the vestibulo-ocular reflex pathway, is comprised of both excitatory and inhibitory components.

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752.8

DISTRIBUTION OF THE CALCIUM-BINDING PROTEINS IN THE VESTIBULAR NUCLEI OF THE GUINEA PIG. S. Saussez, L. Ris, P. Mettens*, N. Gerrits, E. Godaux and R. Pochet. Lab. Histologie, U.L.B., 1070 Bruxelles, Belgium and Lab. of Neurosciences, Univ. of Mons-Hainaut, 7000 Mons, Belgium.

In this study, we have undertaken a detailed mapping of calmodulin, calbindin D28k, calretinin and parvalbumin in the vestibular nuclei of the guinea pig.

Serial 10 µ transverse paraffine sections, either stained by cresyl violet, either immunostained (polyclonal antibodies) using peroxidase-antiperoxidase procedure and diaminobenzidine were analysed.

No neurons were parvalbumin positive. In the superior vestibular nucleus, 80 % of the neurons were calmodulin positive and were uniformely distributed. 30 % of the neurons were calretinin positive without any particular spatial distribution. The 3.5 % of the neurons which were calbindin positive were preferentially located in the dorsolateral part of the nucleus. In the medial vestibular nucleus, 70 % of the neurons were calmodulin positive and uniformely distributed. 32 % were calretinin positive. 9 % were calbindin positive and located along the medial side of the nucleus. In the lateral vestibular nucleus, 90 % of the neurons were calmodulin positive. Giant, medium and small-sized neurons were equally labelled. 31 % were calretinin positive. Among them, 35 % belonged to the giant or Deiters neurons. 3 % which were calbindin positive were of small size. In the descending vestibular nucleus, 90 % of the neurons were calmodulin positive. 14 % were calretinin positive. 10 % were calbindin positive.

We conclude that, in vestibular neurons, calmodulin is highly expressed, calretinin and calbindin are less abundant and parvalbumin is absent.

752.9

SPATIOTEMPORAL PROPERTIES OF CENTRAL OTOLITH NEURONS IN DEVELOPING RATS. C.H. Lai and Y.S. Chan (SPON: The Hong Kong Society of Neurosciences). Department of Physiology, Faculty of Medicine, The University of Hong Kong, Sassoon Road, Hong Kong.

To examine the postnatal maturation of the spatiotemporal properties of vestibular nuclear neurons, extracellular activities were recorded from neurons in the lateral and descending vestibular nuclei of decerebrate rats (7 - 21 days and adult) during off-vertical axis rotations (OVAR: 1.75 - 15°/s; 10° tilt) that selectively stimulate the otoliths. Two groups of units were identified based on their bidirectional response sensitivity, δ, to OVAR in the CW and CCW directions. One group had symmetric and velocity-stable δ; their gain tuning ratios, σ, were similar to those of narrowly spatiotemporal-tuned neurons. The other group had asymmetric and velocity-variable δ; their σ values were similar to those of broadly spatiotemporal-tuned neurons. The proportion of broadly tuned neurons in the sampled population decreased with age: 50% at 7 days of age and 27% in adult. Both the best response orientations of the narrowly tuned units and the preferred orientations of the broadly tuned units were distributed predominantly in the transverse direction in 7-day old rats but were found in all directions on the plane of rotation in older rats. There was also a significant increase in the response gain of these neurons in older rats. Our results suggest that a coordinate frame of head positions with respect to gravity progressively emerges within the vestibular nucleus in the course of postnatal development.

752.10

FUNCTIONAL CHARACTERISTICS OF IPSILATERAL AND CONTRALATERAL INPUTS TO CENTRAL OTOLITH NEURONS OF CATS AFTER ACUTE UNILATERAL LABYRINTHECTOMY. Y.S. Chan*. Department of Physiology, Faculty of Medicine, The University of Hong Kong, Sassoon Road, Hong Kong.

To determine the nature of the ipsilateral and contralateral otolith inputs, extracellular activities of vestibular nuclear neurons on both the lesioned and labyrinth-intact sides were examined during off-vertical axis rotations (OVAR: 1.75 - 15°/s; 10° tilt) in decerebrate cats after acute hemilabyrinthectomy (HL). A bidirectional response sensitivity ratio δ (the sensitivity of each neuron to OVAR in the CW and CCW directions) was used to evaluate the spatiotemporal properties of the neurons. Those with symmetric and velocity-stable δ were grouped as narrowly spatiotemporal-tuned neurons while those with asymmetric and velocity-variable δ were grouped as broadly tuned neurons. More broadly tuned neurons were found in HL cats (a higher proportion on the lesioned side than on the intact side) than in control cats. This suggests that the more prominent broadly tuned responses of HL cats are normally masked by inputs from the contralateral side. On both sides, the directional preference of the broadly tuned neurons and the best response orientations of the narrowly tuned neurons were found predominantly on the segment of the polar diagram ipsilateral to the side of recording, implying that the crossed otolithic inputs carry information to complement inputs arising from the ipsilateral side. Thus, otolith inputs from the two sides contribute to determine the spatiotemporal properties of the vestibular nuclear neurons in coding head orientations. (Supported by H.K. Research Grants Council.)

752.11

RESPONSE OF MEDIAL VESTIBULAR NUCLEUS NEURONS TO HORIZONTAL LINEAR AND ANGULAR STIMULATION OF DECEREBRATE CAT. R.H. Schor*, B.C. Steinbacher, B.J. Yates. Dept. Otolaryngology, University of Pittsburgh, Pittsburgh, PA 15213.

Responses arising from the vestibular utriculus encode the direction of linear accelerations in the horizontal plane. Afferents optimally encoding all directions of tilt exist in roughly comparable numbers, especially if considering inputs from both labyrinths. Yet studies of central vestibular neurons located in the lateral and inferior vestibular nuclei, as well as neurons located in the pontomedullary reticular formation, showed a preponderance of neurons responding more vigorously to roll (lateral tilt) than to pitch (fore-aft tilt).

Guided by observations that lesions that include the medial vestibular nucleus (MVN) abolishes (pitch) vestibulo-sympathetic reflexes, we examined the responses of neurons in the MVN and adjacent inferior vestibular nucleus (IVN) of 7 decerebrate cats. Responses attributed to activation of otolith (utricular) receptors were recorded during constant velocity rotation in the animal's horizontal plane about an axis tipped 10° from the vertical (changing linear acceleration). Out of 47 tested neurons, 28 responded to this stimulus; 12 responded best when nose down or nose up, 5 responded best when one ear was down, and the other 11 responded best at intermediate positions near the planes of the vertical canals.

Many of these responsive neurons were also tested for horizontal canal input using sinusoidal rotation (angular acceleration) about an earth-vertical axis. 25/45 neurons were modulated by this stimulus, with response dynamics consistent with canal input. In 41 neurons tested, 16 responded to both stimuli, suggesting convergence between utricular and horizontal semicircular canal afferents.

These results suggest that in addition to its well-known input from the horizontal semicircular canals, the MVN may serve as an important relay for pitch linear acceleration stimuli. Supported by NIH Grants NS24930 and DC00693.

752.12

CALCIUM-DEPENDENT CONTROL OF SIGNAL TRANSFORMATIONS IN MEDIAL VESTIBULAR NUCLEUS NEURONS. Sascha du Lac*, UCSF Dept. of Physiology and Keck Center for Integrative Neuroscience, San Francisco CA 94143.

A combination of mechanisms at the cellular and network levels determine how head movement signals are transformed into the appropriate oculomotor commands. To investigate the cellular control of signal transformations in vestibulo-ocular reflex pathways, we have assessed the effects of blocking calcium channels on the gain and dynamics of spike generation in Medial Vestibular Nucleus (MVN) neurons. MVN neurons recorded in avian brain slices were injected with intracellular current to measure spike generation gain (slope of the relationship between mean firing rate and input current amplitude) and dynamics (timecourse of instantaneous firing rate during the step).

In normal MVN neurons, spike generation is highly linear over a wide range of input amplitudes and has highly stereotyped dynamics including little decrease in firing rate during the course of a 1 sec current step. Bath application of the calcium channel blocker cadmium (Cd; 100 uM) had a number of pronounced effects on spike generation in MVN neurons. First, the relationship between current and firing rate became nonlinear in the presence of Cd: a given amount of current produced smaller changes in firing rate as the current amplitude increased. Second, spike generation gain increased, relative to control, at low current amplitudes. Finally, Cd altered spike generation dynamics: firing rate decreased markedly during current steps. The small conductance calcium-activated potassium (SK) channel can not be solely responsible for these effects: blocking the SK channel with apamin (100-200 nM) produced a smaller effect on spike generation gain with no effects on linearity or dynamics. These results demonstrate that calcium influx into MVN neurons modulates spike generation gain, dynamics, and linearity and suggest that regulation of calcium-dependent processes could underlie adaptive changes in the gain and dynamics of the vestibulo-ocular reflex.

752.13

METABOTROPIC GLUTAMATE RECEPTORS IN THE MEDIAL VESTIBULAR NUCLEUS IN VITRO. P.F.Smith*, C.L. Darlington Dept.of Psychology, Univ. Otago, Dunedin, New Zealand

The aim of the present study was to examine the response of guinea pig medial vestibular nucleus (MVN) neurons in brainstem slices to the selective metabotropic receptor agonist, 1S,3R-amino-cyclopentyl-1,3-dicarboxylate (ACPD). Extracellular recordings were made from single MVN neurons using standard in vitro techniques. 60% (12/20) of MVN neurons responded to 1S,3R-ACPD at a concentration of 10^{-6} M, compared to 40% (8/20) and 35% (7/20) of neurons at concentrations of 10^{-8} and 10^{-10} M. Both increases and decreases in firing rate were observed in different neurons: in general, the magnitude of the responses was large and the duration of the responses was long. Recordings from a slice which contained only the MVN confirmed that these responses were produced by the action of the metabotropic agonist within the MVN itself.

752.14

EFFECT OF VESTIBULOCEREBELLUM AND INTERACTION OF NECK INPUT WITH VESTIBULAR INPUT TO THE CAUDAL VESTIBULAR NUCLEI. D. B. Thomson* H. Ikegami, R. H. Schor and V. J. Wilson. The Rockefeller University, New York, NY, 10021.

Previously, we studied the vertical vestibular input to neurons in the caudal half of the descending and medial vestibular nuclei (CVN) in decerebrate cats with the caudomedial cerebellum removed. In the present study we investigated whether CVN neurons respond differently to vestibular stimuli when the caudal cerebellar vermis is intact. Little influence was observed. As in the partially decerebellated preparation, neurons classified as receiving input primarily from otoliths displayed, on average, a modest gain increase (<threefold per decade) and phases that led position by 33-39° over the range of 0.05-1 Hz whereas canal neurons displayed a gain slope of 19 per decade and phases that led velocity by more than 30°. In both preparations, the response vector orientations of canal neurons were clustered near the canal planes and those of otolith neurons tended to be in the roll quadrants. The CVN receive direct projections from upper cervical primary afferents, and we further examined the responses of neurons in this region to natural neck rotation and to combined vestibular-neck stimulation. Only 30% of neurons responded to neck rotation in the range of 0.05-0.5 Hz. We investigated neck-vestibular interaction in 17 neurons that showed neck and vestibular responses with phase differences <45°. Only 7/17 neurons had "antagonistic" neck and vestibular vector orientations (i.e. differing by >120°) and 3/17 neurons had "synergistic" vectors (i.e. differing by <60°). In contrast, >50% of neurons in more rostral areas of the vestibular nuclei receive neck input, which is almost always antagonistic. Supported by NIH (DC 02187 and NS 24930) and by HFSPO.

752.15

DYNAMIC RESPONSES OF VESTIBULAR NUCLEI AND PREPOSITUS NEURONS IN THE ALERT GERBIL. G.D. Kaufman* and A.A. Perachio. Departments of Otolaryngology, Physiology & Biophysics and Anatomy & Neurosciences, Univ. TX Med. Br., Galveston, TX 77555-1063.

The responses of neurons in the nucleus prepositus hypoglossi (PrH) were recorded in alert gerbils during horizontal angular or linear head acceleration in the dark. Angular acceleration, ranging from 0.05 to 2.0 Hz, 30°/sec. to 120°/sec., was applied in the plane of the horizontal semicircular canals. At frequencies below 0.5 Hz, phasic bursting occurred during the otherwise inhibitory portion of the cycle in some PrH neurons. From 0.2-2.0 Hz gain increased (0.23 S/s/deg/s ± 0.04 SEM to 0.43 S/s/deg/s ± 0.07 SEM). Response phase led 23° ± 7° SEM at 0.2 Hz. Phase gradually decreased with frequency. Above 0.5 Hz phase lag increased to 26° ± 4° SEM at 2.0 Hz. Otolith organ-related responses were recorded to pure linear acceleration during horizontal sinusoidal translational motion, ranging from 0.25 to 5.0 Hz, 0.05 to 0.5 x g peak accelerations. Average response gain at 0.5 Hz and 0.1 G was 194 S/s/g ± 26 SEM. Phase led acceleration 110° ± 26° SEM. Two-dimensional spatio-temporal convergence was observed in most cells for frequencies above 0.25 Hz. In contrast to medial vestibular nuclei (MVN) cells, the response vector maximum sensitivity in PrH neurons phase led acceleration by 90° while the orthogonal vector produced an acceleration phase related response. A model will be presented of the interaction of PrH and MVN neurons at abducens motoneurons to regulate vestibulo-ocular responses. Supported by NIH grants DC00385 and DC00111.

752.16

VESTIBULAR NUCLEI NEURON RESPONSE DYNAMICS PRODUCED BY COMMISSURAL CONVERGENCE IN PIGEONS. J.D. Dickman*. Depts. of Surgery (Otolaryngology), Anatomy, and Physiology, University of Mississippi Medical Center, Jackson MS 39216

How do vestibular nuclei neurons synthesize the information from the paired complementary semicircular canals into a unified output signal? To answer this question, the dynamic properties of vestibular nuclei neurons in pigeons were examined by individual and paired stimulation of the horizontal semicircular canals using both mechanical micropushers and rotational acceleration. Extracellular single fiber responses from horizontal canal-related neurons were obtained in awake decerebrate birds that were paralyzed (Pancuronium) and ventilated (250 ml/min, O_2/CO_2). Mechanical micropusher stimulation (0.01 to 10 Hz, ±2.5 μm) of the exposed ipsilateral horizontal membranous duct was delivered first, followed by identical contralateral canal stimulation. Bilateral mechanical stimulation was next delivered, with left and right canal stimuli being 180° phase displaced. Rotational stimulation (0.01 - 4 Hz, 20 deg/sec) was finally delivered as a comparison. All Type I neurons responded to ipsilateral canal stimulation with increasing gains and phase advances relative to inward membranous duct displacement. Contralateral canal stimulation produced responses with much small gains and phases that were generally phase displaced by 180°. Two different response patterns were obtained with bilateral canal stimulation; one with additive gains from ipsilateral and contralateral canal responses and one with subtractive gains. Phase differences in the ipsilateral and contralateral canal high frequency stimulation were correlated with the type of response profile, where differences greater than 180° resulted in subtractive gains. These phase differences suggest that spatio-temporal convergence between inputs from the two complementary labyrinths exist for vestibular nuclei neurons and may be utilized to produce the total output signal that is projected to the target cell.

752.17

RESPONSES OF SECONDARY VESTIBULAR NEURONS TO MECHANICAL VESTIBULAR STIMULATION IN THE TOADFISH *OPSANUS TAU*. A. F. Mensinger* and S. M. Highstein, Dept. of Otolaryngology. Wash. Univ. Sch. of Med., St. Louis, MO 63110.

The response dynamics of toadfish horizontal semicircular canal afferents have been classified into three rough groups (high- and low-gain velocity sensitiveand acceleration; Boyle & Highstein 91, Rabbit *et al.* 95) utilizing rotary and mechanical stimuli. We employed mechanical stimuli (canal indentation) to study the fidelity of transfer of these nerve dynamics to central afferent branches and secondary vestibular neurons. Intra- and extracellular recording and intracellular injection of recorded neurons were utilized. The central branches of primary afferents were labeled and identified by the presence of terminal boutons and nerve trunks traveling peripherally. In general, responses of afferent central branches were 2-5 times less sensitive and phase-lagged control peripheral records. Secondary neurons, primarily in the descending and posterior octaval nuclei demonstrated similar response dynamics as central branches. Both high and low gain responses were observed but phase covered a broader range than in primary afferents. In contrast to peripheral nerve responses, central neurons were insensitive to low frequency stimuli (below 1Hz) but were responsive in the 1-10 Hz range These results suggest that central processes of primary afferents serve to modify response dynamics of peripheral semicircular canal nerve axons.

752.18

RESPONSES OF VESTIBULAR NUCLEUS TO ROTATION OF THE TURTLE HEAD *IN VITRO*. M. Ariel*, T.X. Fan and C.A. Scudder. Saint Louis Univ., MO 63104 and Univ. of Pittsburgh, PA 15213.

Extracellular spike recordings were made from the vestibular nucleus (VN) during rotation of an *in vitro* turtle brainstem in which the temporal bones remained attached. The lateral canals were roughly aligned with the horizontal plane and the vertical axis of rotation was centered between the labyrinths. This preparation responded to vestibular stimulation for up to several days. In some preparations, horizontal canal sensitivity was confirmed by plugging the vertical canals or stimulation about the interaural axis.

Single VN units were isolated when they responded to horizontal sinusoidal stimulation and then quantified for different stimuli. Spike histograms were averaged from many stimulus cycles and fit to a rectified sine function. VN units were then categorized as encoding stimulus velocity or acceleration in the ipsi- or contraversive direction.

Common features of these neurons were their low levels of spontaneous activity and their rectified sinusoidal responses to sinusoidal rotation. Each cell had a characteristic phase (re table velocity) that was invariant below 1 Hz stimulation. Response phase varied substantially between cells, with most phase values between a 45° lag to a 90° lead. The response gain (re table velocity at 1 Hz) was between 0.1 and 25, indicating substantial heterogeneity of these cells' responses. These response features will be described and compared to that of turtle vestibular afferents and VN cells in other species. (Supported by EY 05978 to MA)

752.19

CERVICAL INFLUENCES ON THE DIRECTIONAL PROPERTIES OF THE VESTIBULOSPINAL REFLEX. O.Pompeiano*, P.Andre and D.Manzoni. Dept. Physiol. Biochem., Univ. of Pisa, Via S.Zeno 31, Pisa, I-56127.

The vestibular input induced by rotation of the animal in a given direction produces an appropriate postural adjustement. We investigated whether the pattern of the vestibulospinal (VS) reflex recorded from the forelimb extensor triceps brachii (TB) could be modified by a relative body-to-head displacement, as expected in order to preserve body stability. In decerebrate cats, the multiunit EMG activity of the TB was recorded during wobble of the whole animal at 0.15 Hz, 10°. This stimulus allowed to determine the muscle response vector corresponding to the direction of head displacement leading to the maximal EMG response. When the body was kept straight with respect to the head the response vector of the TB was always oriented close to the transverse axis, pointing to the side-down direction. Following 30° of body-to-head displacement around a vertical axis passing through the atlanto-occipital joint, the response vector of the TB shifted in the same direction of body rotation, thus remaining approximately perpendicular to the body axis. The shift of the response vector was consistently reduced by inactivation of the cerebellar anterior vermis following microinjection in lobule V (culmen) of the GABA-A agonist muscimol (0.5 μl at 8 μg/μl saline). These findings indicate that: i) the sensory input of cervical origin is able to modify the pattern of the VS reflex which appears to be organized in a body-centered reference frame; ii) the cerebellar vermis is required for the proper execution of this sensorimotor transformation.

752.20

DIFFERENCES BETWEEN THE UTRICULAR ACTIVATED AND SACCULAR ACTIVATED SECOND ORDER VESTIBULOSPINAL NEURONS IN CATS. H. Sato[1*], M.Imagawa[1], M. Sasaki[1], K. Endo[1], H. Ikegami[1], N. Isu[2] and Y. Uchino[1]. [1]Dept. of Physiol., Tokyo Med. College, Tokyo 160; [2]Dept. of Inform. Eng., Tottori Univ., Tottori 680.

Axonal trajectories and locations of the cell bodies of utricular (UT) and saccular (SAC) activated vestibulospinal neurons (VSNs) were studied in decerebrated or anaesthetized cats. Bipolar stimulating electrodes were placed on either the UT or the SAC nerve with the other vestibular nerve branches transected. Monopolar stimulating electrodes were positioned in the lateral vestibulospinal tracts (LVST) bilaterally and the medial vestibulospinal tracts (MVST) at the C1. Similar electrodes were bilaterally inserted into the ventral funiculi at the C2-4, C7-T1 and L3, and into the third nucleus. The majority of UT-activated VSNs were located in the rostral part of the descending vestibular nucleus, while the SAC-activated VSNs were mainly located in the lateral vestibular nucleus. Almost none of UT- and SAC-activated VSNs were activated from the third nucleus. The numbers of UT-VSNs (n=46) and SAC-VSNs (n=46) activated from the each spinal segment were as follows:

Spinal segments		C1	C2-4	C7-T1	L3
i-LVST	utricular	32	30	28	7
	saccular	14	12	8	3
MVST	utricular	6	4	1	0
	saccular	29	20	10	0
c-LVST	utricular	8	5	0	0
	saccular	3	1	0	0

OCULOMOTOR SYSTEM: BRAINSTEM AND PRETECTUM

753.1

ANATOMICAL SUBSTRATES FOR PRETECTAL CONTROL OF LID MOVEMENTS IN A PRIMATE. P.J. May* Deparments of Anatomy and Neurology, University of Mississippi Medical Center, Jackson, MS 39216.

The eyelids are controlled by two voluntary muscles. The levator palpebrae superioris, whose motoneurons are found in the caudal central subdivision of the oculomotor complex, is active during vertical eye movements, while the orbicularis oculi, whose motoneurons are found in the facial nucleus dorsolateral subdivision, produces blinks. Based on clinical and non-primate experimental evidence, the possibility of pretectal inputs controlling lid movements was investigated by injecting WGA-HRP, biocytin or biotinylated dextran amines (BDA) into the pretectum of macaque monkeys. While large pretectal injections bilaterally labelled terminals in both the orbicularis and levator subdivisions, a smaller WGA-HRP injection centered in the olivary pretectal nucleus did not label terminals in these subdivisions. Nevertheless, a similar olivary biocytin injection did reveal a sparsely distributed collection of fine terminals and boutons among retrogradely labelled levator motoneuons. These were most dense along the ipsilateral edge of the caudal central subdivision. BDA injections that included the nucleus of the posterior commissure bilaterally labelled thick axonal arbors in the caudal central subdivision that had numerous large terminal and *en passant* puncta. Similar terminal arbors were also observed bilaterally among the orbicularis oculi motoneurons in a case with a caudally placed large nucleus of the posterior commissure injection. However, these facial terminals were not present in a case where only the nucleus of the posterior commissure and the anterior pretectal nucleus were involved. A BDA injection in the superior colliculus and posterior pretectum also produced arbors with large puncta in the caudal central subdivision. In conclusion, the light evoked blink reflex pathway that connects the olivary pretectal nucleus to orbicularis oculi motoneurons in both facial nuclei in the cat is not evident in the monkey, although a minor, perhaps inhibitory projection to levator motoneurons is present. A pretectofacial pathway is present, but its precise source remains to be identified. Another pretectal area, the nucleus of the posterior commissure, may supply a major input to levator motoneurons. This projection may modulate lid movements in concert with vertical gaze changes. Support: EY09762.

753.2

THE PIGEON ACCESSORY OPTIC SYSTEM: VISUAL INPUT IN EYE MUSCLE COORDINATES D.R. Wylie*, R.G. Glover, K.L. Lau, B. Morgan, and B.J. Frost. Department of Psychology, University of Alberta, Edmonton, Alberta, Canada, T6G 2E1.

The generation of compensatory eye movements in response to rotational head movements involves the transformation of visual-optokinetic and vestibular signals into commands controlling the appropriate eye muscles. The optokinetic information is analysed by the Accessory Optic System (AOS). It has been demonstrated in rabbits and pigeons that the three systems (AOS, vestibular, eye muscle) share a *similar* three-dimensional reference frame. In this report we suggest that in pigeons the optokinetic system is organized with respect to the eye muscles rather than the vestibular canals. Measurements of the plane of the horizontal recti were obtained from 5 pigeon eyes. Using standard extracellular techniques we recorded from two structures in the pigeon AOS: (1) the pretectal nucleus lentiformis mesencephali (LM), and (2) the nucleus of the basal optic root (nBOR). The LM and nBOR contain neurons responsive to optokinetic stimuli (OKS) moving forward and backward in the contralateral hemifields, respectively. Thus, activity in the LM and nBOR result in contraction of the contralateral medial rectus (MR) and lateral rectus (LR), respectively. We also recorded from Purkinje cells in the flocculus of the vestibulocerebellum (VbC). Neurons in the VbC have binocular receptive fields (RFs). The ipsilateral and contralateral RFs respond to OKS moving forward and backward, respectively. Thus, activity of VbC neurons results in contraction of the ipsilateral MR and contralateral LR, respectively. A close correspondence was found between the plane of the MR, the direction preferences of LM neurons, and VbC neurons in response to stimulation of the ipsilateral hemifield. In all cases the vectors were approximately aligned with the horizontal. A close correspondence was also found between the plane of the LR, the direction preferences of nBOR neurons, and VbC neurons in response to stimulation of the contralateral hemifield. In all cases the vectors were tilted downward from the horizontal by about 30°. In sum, there is a close correspondence between the planes of the horizontal eye muscles and the direction preferences of AOS and VbC neurons providing optokinetic input to those muscles.

753.3

EFFECTS OF MESENCEPHALIC RETICULAR FORMATION (MRF) ELECTRICAL MICROSTIMULATION UPON EYE MOVEMENTS IN PRIMATES. V.L. Silakov*, D.M. Waitzman and S.J. Tinturin. Newington VAMC, 555 Willard Ave, Newington, CT 06111.

While electrical microstimulation (MCS) is known to generate contraversive horizontal eye movements (EM) in monkeys (Cohen et al., 1984), the effects of behavioral state, variation in the rostral-caudal site, and changes in the initial position (IP) of the eyes have not been systematically explored. EMs were elicited using 80-100 µamps of current through tungsten microelectrodes in two head fixed monkeys. The monkeys sat in a dark room and fixated a small spot of light which either remained lit or was extinguished just prior to MCS. Sixty sites throughout the rostral-caudal extent of the MRF were explored. At 23 sites IP was varied across a 5 or 9 point grid which spanned the oculomotor range in 20° steps. Saccades from 0.5° to 25° amplitude were elicited. Direction of elicited EMs varied from primarily horizontal in the caudal regions to vertical in the more rostral regions. At 60% of the sites at which IP was varied, there was a distinct correlation of IP with elicited saccade amplitude ($r^2 > 0.5$) such that shifts of IP toward the direction of the movement (as defined from primary position) were associated with smaller elicited amplitudes. A commensurate change in saccade direction accompanied the reduction in saccade amplitude. The large changes in EMs following MCS suggest that the MRF, distinct from the superior colliculus, may be organized in a craniotopic coordinate system.

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753.4

CELLS ENCODING VELOCITY AND DYNAMIC MOTOR ERROR RESIDE IN THE MONKEY OCULOMOTOR COMLEX (III) AND MESENCEPHALIC RETICULAR FORMATION (MRF).
D.M. Waitzman*, V.L. Silakov and D.R. D'Angelo. Newington VAMC, 555 Willard Ave, Newington, CT 06111.

Previous single unit recordings in III have emphasized that the pulse of activity observed in oculomotor neurons (OMNs) is stereotypic and the frequency of the instantaneous discharge during the OMN pulse controls eye velocity. The discharge dynamics of 43 neurons within III and 31 horizontal, saccade related MRF neurons were studied in 2 awake, head-fixed monkeys performing visually guided saccades. Neuronal discharge frequencies were expressed as a spike density function (SD) and individually matched to the temporal dynamics of individual saccades. Motor error (ME) neurons (MRF=8 and III=12) had a monotonic decline of SD with declining motor error. Phase plane plots of SD versus eye velocity generated an open loop indicating a different level of activation during the accelerating and decelerating phases of the saccade. Velocity (VEL) neurons (MRF=16 and III=11) had a "ski-jump" decline of SD with declining ME. These cells showed a closed loop response for the SD versus VEL phase plane. The remainder of the neurons demonstrated a mixture of these two responses. In light of similar responses of saccade related cells in the superior colliculus, these results suggest that both VEL and ME are controlled variables at multiple levels of the primate oculomotor system.

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753.5

THE NITRIC OXIDE - cGMP PATHWAY CONTRIBUTES TO THE CONTROL OF HORIZONTAL EYE MOVEMENTS BY PREPOSITUS HYPOGLOSSI NEURONS. M. Escudero*, B. Moreno-Lopez, and C. Estrada§. Laboratory of Neuroscience, Faculty of Biology, University of Sevilla, and §Department of Physiology, Faculty of Medicine, UAM, Madrid, Spain.

Nitric oxide (NO) is a difusible gas synthesized by some neurons in the central nervous system, with an intercellular communication function, which is mediated in most cases by activation of guanylate cyclase in target cells. The possible role of nitric oxide as a modulator in the cat oculomotor system was investigated based on the large number of neurons containing NO synthase (NOS) present in the prepositus hypoglossi nucleus (PH).

Alert cats prepared for chronic recording of eye movements by means of the magnetic search-coil technique were used. Local injections of the NOS inhibitors L-nitroarginine methyl ester (L-NAME; 10-40 nmol) and L-N-monomethylarginine (L-NMMA; 30-60 nmol) in the anterior third of the PH produced conjugated nistagmic eye movements with slow phases directed to the contralateral side. This spontaneous eye movement alteration was dose-dependent, appeared within 1-2 minutes after injection, lasted for 30-60 minutes and was more evident in complete darkness. The effect of NOS inhibitors was stereo specific because the isomer D-NAME was without effect, and was abolished by simultaneous administration of the NOS substrate, L-arginine. Injections of the NO donor sodium nitroprusside (SNP) or the permeative analog of cGMP 8-bromo-cGMP in the PH produced an effect opposite to that of NOS inhibitors, this is, conjugated nistagmic eye movements with slow phases directed to the ipsilateral side. These results indicate that NO produced by PH neurons modulates via cGMP the generation of the motor signal that control horizontal eye movements.

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753.6

MODELING THE OCULOMOTOR EFFECTS OF IBOTENIC ACID LESIONS OF THE MACAQUE NUCLEUS PREPOSITUS HYPOGLOSSI (NPH). R. Soetedjo[1,3] and C.R.S. Kaneko*[2,3] Depts. Bioengineering[1] and Physiology & Biophysics[2] and Regional Primate Research Center[3], Box 359010, University of Washington, Seattle, WA 98195-9010

The nature of the effects of lesions of the nph, the putative position integrator in the oculomotor system, was examined by comparing the behavior of a monkey that had multiple bilateral lesions of its nph with predictions of the Scudder and Robinson models. A multiply nph-lesioned monkey cannot hold its eyes on an eccentric target for fixation after making a saccade and position drift in the dark is greatly exacerbated. The shape of the drift showed two components, an initial fast drift followed by a slower decay. In order to fit the shape of the monkey's drift with either model, the lesion must affect both the time constant and gain of the integrator path. If gain and time constants are adjusted to fit drift in the dark, Robinson's model predicts that saccades will overshoot and Scudder's model predicts undershoot both followed by the drift. Robinson's model also predicts a large overshoot if the gain of the integrator is too low.

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753.7

ANALYSIS OF PRIMATE INHIBITORY BURST NEURON SPIKE TRAIN DYNAMICS: GAZE VERSUS EYE BASED MODELS DURING COMBINED EYE-HEAD GAZE SHIFTS. K.E.Cullen* and D.Guitton. Aerospace Medical Research Unit & Montreal Neurological Inst. McGill University, Montreal, Canada.

We used metric analysis and systems identification techniques to relate IBN discharges to the dynamics of gaze, eye, and head during head-free gaze shifts. Burst versus gaze durations were similar head-fixed and head-free, and the number of spikes was better correlated to head-free gaze, than eye amplitude. Latencies calculated using dynamic models were significantly lower than those based on the onset of the first spike in head-fixed and head-free conditions. Regardless of an IBN's onset or dynamic lead, simple downstream (velocity based) models were better than upstream models (non-linear function of motor error) at predicting IBN discharges. The most relevant parameters to head-free downstream models were a velocity gain coefficient and a bias term. For all models with a bias term, gaze and eye velocity were equally good at predicting IBN activity, however the bias term estimated for eye based models was significantly higher than that for gaze based or head-fixed models. Head velocity based models provided worse fits than gaze or eye velocity based models. Acceleration and higher order non-linear velocity terms slightly improved the variance accounted for in our models (10%), whereas, use of acceleration and pole terms (the derivative of the IBN firing rate) greatly improved model fits, when initial conditions (ICs) were estimated as parameters (115%). However, these models yielded bias terms that were unrealistically negative. Models with only a gain and bias term, in which the bias term was estimated separately for each gaze shift, provided fits comparable to those with a pole term with estimated ICs. Estimated biases were inversely correlated with the peak gaze velocity and/or amplitude for 39 % of the IBNs, in both head-free and head-fixed conditions (gaze base models). In contrast, these values were related with peak velocity and/or amplitude of the head-free eye movement component for only 18% of IBNs (eye base models). We suggest that the bias term is critical for evaluating the significance of model fits: 1) the bias enables a distinction between head-fixed and head-free fits, and suggests that gaze velocity is more relevant than eye velocity in describing IBN discharges during gaze shifts 2) the bias is strongly related to the metrics of saccades head-fixed and the metrics of gaze, not eye, head-free.

753.8

NEURAL NET SIMULATION OF EYE POSITION COMMAND TO MEDIAL RECTUS MOTONEURONS FROM ABDUCENS INTERNUCLEAR NEURONS. Paul Dean*, Dept. of Psychology, University of Sheffield, Sheffield S10 2TP, England.

Ocular motoneuron firing rate is linearly related to eye position (in the relevant direction) with slope K, above recruitment threshold T. Within the population of ocular motoneurons K increases as T increases. It is not known how these relations are derived from the combination of input signals with intrinsic motoneuron properties.

Possible derivations were investigated by simulating the input signal to medial rectus motoneurons (MR-MNs) from internuclear neurons of the abducens nucleus (AINs). AINs were represented as input nodes in a two layer neural-net, each with weighted connections to every output node representing an MR-MN. The output node bias term acted as the intrinsic MR-MN threshold. Inputs to the net were conjugate eye-position commands, used to generate realistic firing patterns in the AINs (Fuchs et al., *J.Neurophysiol.* 60:1874, 1988; Gamlin et al., *J.Neurophysiol.* 62:70, 1989). The output of the net was compared with actual MR-MN firing patterns for that eye position (Gamlin & Mays, *J.Neurophysiol.* 67:64, 1992). Weights were adjusted as a result of the comparison using gradient descent error-reduction.

The simulations showed: (1) MR-MN firing rates were accurately reproduced by AIN populations in which K was unrelated to recruitment threshold T, and in which maximum values of T were up to 25 deg less than those in the MR-MN population. (2) Accuracy could still achieved when the bias term, representing MR-MN intrinsic threshold, was held constant. (3) Weights between AINs and MR-MNs showed that each MR-MN typically received functional connections from a cluster of AINs with similar Ts.

It is therefore possible that appropriate patterning of input connections can determine MR-MN firing rates in relation to conjugate fixation commands, without a major contribution from variation in intrinsic motoneuron properties.

753.9

EXTRAOCULAR MUSCLE DEVELOPMENT IN A MODEL OF MIDBRAIN DYSGENESIS. J.D. Porter* and R.S. Baker. Depts. of Anatomy & Neurobiology and Ophthalmology, U. of Kentucky Med. Ctr., Lexington, KY 40536.

The relative importance of genetic and epigenetic factors in developmental regulation of the unique extraocular muscle (EOM) phenotype is unclear. The *Wnt-1* allele is required for the development of the mesencephalon, including the oculomotor and trochlear nuclei. *Wnt-1* mutant mice, by homologous recombination (McMahon and Bradley, *Cell* 62:1073, '90), then may represent a serendipitous model in which to examine the role of neuromuscular interactions in developing EOM. Prior studies have shown that the EOM primordia are innervated prior to migration away from their somitomeric origins. However, innervation may not be a critical factor in guidance of migration since *Wnt-1* mutants showed normal spatial orientation of the six EOMs within the orbit at E14.5-16.5 in spite of the absence of oculomotor and trochlear motoneurons. Lateral rectus muscles contained axons and primitive neuromuscular contacts from the intact abducens motoneurons. However, some of the muscles normally innervated by oculomotor and trochlear nuclei received motor innervation from an alternative source, presumably via collateral sprouting of abducens motoneurons. Muscles receiving either normal or aberrant innervation exhibited myogenic staging comparable to control littermates. By contrast, muscles lacking evidence of innervation contained atrophic and degenerating myotubes. The pattern of pathology in aneural muscles suggests that innervation from oculomotor nerves plays a role in both primary and secondary myogenesis in this muscle system. Taken together, this model may allow characterization of the mechanisms responsible for the tissue-specific properties of the EOM allotype. The pattern of developmental aberrant innervation of EOMs seen in this mouse also may provide insight into the etiology of Duane retraction syndrome in humans. Supported by NIH EY09834 and Research to Prevent Blindness.

753.10

THE PRETECTAL NUCLEUS OF THE OPTIC TRACT (NOT) SUBSERVES LATENT NYSTAGMUS IN VISUALLY DEPRIVED MONKEYS. M.J. Mustari[1]*, A.F.Fuchs[2], R.J. Tusa[3], A. Burrows[1], and C. Livingston[1]. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX[1]; Regional Primate Research Center, University of Washington, Seattle, WA.[2], University of Miami Ear Institute, Miami, FL[3].

Monkeys (Macaca mulatta) binocularly deprived (BD), by lid suture, early in life, develop a permanent horizontal nystagmus, resembling latent nystagmus (LN)[1]. These animals also lack a nasal-to-temporal optokinetic reflex (OKN). To discover the locus for LN, we recorded from and reversibly inactivated the NOT, which is essential for horizontal optokinetic eye movements. We recorded NOT unit activity during spontaneous nystagmus and fixation, and evaluated visual receptive fields in two trained monkeys (> 2 yr old), which had been BD for the first 25 or 55 days of life. NOT units in BD animals were either driven exclusively (55 day BD) or were dominated (62%; N=32) by the contralateral eye (25 day BD). This is in contrast to normal monkeys where all NOT units are binocularly driven[2]. In addition, most NOT units changed their spontaneous firing rates as a function of ambient light levels. Such light intensity dependent firing, together with contralateral ocular dominance, could be the source of an unbalanced drive to caudal brainstem structures, resulting in LN.

To test this suggestion, we placed injections of the GABAa agonist, muscimol (0.3µl; 2%) in the NOT. Prior to muscimol injection, LN was initiated 145ms (±15) after closing a shutter in front of the right eye, with rightward slow phase eye velocity reaching 40°/s within a few seconds. Following unilateral muscimol blockade of the NOT, ipsiversive (slow phase) LN due to occluding the ipsilateral eye was completely abolished. Similarly, bilateral NOT blockade abolished LN in either direction. Horizontal optokinetic eye movements were virtually abolished following such bilateral injections, however, the monkey's ability to fixate or make saccades was preserved.

The results of our studies indicate that the NOT is critical for the production of LN in animals subjected to brief periods of visual deprivation early in life. [1]Tusa et al., Invest. Ophth. Vis. Sci. 32: 134-141, 1991. [2]Mustari and Fuchs, J. Neurophysiol. 64:77-90, 1990. Supported by: EY06069, EY09289, RR 00166, EY00745.

753.11

THE PARAFASCICULAR NEURONS IN AND AROUND THE MEDIAL LONGITUDINAL FASCICLE WHICH PROJECT TO THE CEREBELLAR FLOCCULUS AND VENTRAL PARAFLOCCULUS RECEIVE AFFERENTS FROM THE VESTIBULAR NUCLEI IN THE MONKEY. N.M. Gerrits, B. Baggerman, G. Cheron and M. Godschalk*. Dept. of Anatomy, Erasmus Univ., Rotterdam, The Netherlands and Dept. of Neurophysiol., Univ. of Mons-Hainaut, Belgium.

Judged by the number of neurons that can be visualized with retrograde transport of axonal tracers, the dorsomedial pontine reticular formation is one of the major sources of mossy fibers to the flocculus and ventral paraflocculus. Several studies in rat, cat, rabbit and monkey demonstrated in and around the mlf a population of so-called paramedian tract neurons (PMT) with a size similar to that of the population of "secondary" vestibulo-cerebellar mossy fiber neurons. In the monkey (M. fascicularis), approximately 40 percent of the PMT's is located in two clusters, one ± 2 mm caudal to the facial genu, the other directly rostral to the abducens nucleus. The majority of PMT neurons are dispersed between the bundles of the mlf.

Injections with WGA-HRP in the medial (MV) and superior (SV) vestibular nuclei resulted in wide-spread labeling of axon terminals in the entire region containing PMT neurons. Since the ascending connections of the MV and SV are basically related to horizontal and vertical eye movements (EM), respectively, the results suggest an intermingling of horizontal and vertical EM related PMT neurons.

753.12

SYNAPTIC ACTIONS OF VERTICAL EYE POSITION-RELATED INTERSTITIOVESTIBULAR NEURONS IN THE ALERT CAT. Y. Iwamoto*, S. Chimoto, E. Nambu, K. Yoshida Dpt. Physiology, Inst. Basic Med. Sci., Univ. of Tsukuba, Tsukuba, 305 Japan

We have recently shown that many vertical eye position-related neurons in and around the interstitial nucleus of Cajal (INC) project to and arborize in the ipsilateral superior vestibular nucleus. In the present study, we investigated in the alert cat possible projections of vertical eye position-related INC neurons to the trochlear nucleus. In two animals, 37 of 113 neurons were activated antidromically from the trochlear nucleus or nearby MLF. Most activated neurons (33/37) had downward on-directions. We performed tracking through the trochlear nucleus to obtain the depth profile of thresholds for antidromic activation. Multiple low threshold peaks and variation in latency were observed in some neurons with downward on-directions, suggesting direct projection of downward-on INC neurons to trochlear motoneurons. Since interstitiovestibular neurons also had downward on-directions, there is a possibility that the same downward-on INC neurons project to both the trochlear and vestibular nuclei. We then made an attempt to decide the synaptic nature of interstitiovestibular neurons by spike-triggered average of field potentials in the superior vestibular nucleus. In three eye position-related neurons with downward on-directions, unitary synaptic field potentials with a negative polarity were detected at monosynaptic latencies, suggesting excitatory actions. The results suggest that vertical eye position-related interstitiovestibular neurons exert excitatory synaptic actions upon both vestibular nucleus neurons and trochlear motoneurons.

753.13

EFFERENT PROJECTIONS OF THE PRIMATE INTESTITIAL NUCLEUS OF CAJAL. A.K. Moschovakis*, C.A. Scudder[1], C. Balaban[1], S.M. Highstein[2] and T. Kokkoroyannis. Laboratory of Neurophysiology, Dept. of Basic Sciences, P.O. Box 1393, Faculty of Medicine, University of Crete, Iraklion 71110, Grete, Greece, [1]Eye and Ear Institute, Pittsburgh University, Pennsylvania, U.S.A., and [2] Department of Otolaryngology, Washington University School of Medicine in St. Louis, Missouri, U.S.A.

The efferent projections of the mesencephalic Nucleus Interstitialis of Cajal (NIC) were studied in the squirrel monkey following bulk injections of biocytin and PHA-L near functionally identified oculomotor related burst-tonic neurons of the NIC. Dense terminal fields were encountered: a) contralaterally, in the NIC, the oculomotor nucleus and the trochlear nucleus, and b) ipsilaterally, in the Fields of Forel, the rostral interstitial nucleus of the medial longitudinal fasciculus, the oculomotor and trochlear nuclei, the gigantocellular reticular formation, as well as the ventromedial and commissural nuclei of the first two cervical segments of the spinal cord. Moderate or weak terminal fields were observed: a) bilaterally, in the mediodorsal, centre medianum central lateral, central medial and parafascicular thalamic nuclei, and b) ipsilaterally, in the zona incerta, the mesencephalic reticular formation, the pedunculopontine nucleus, the nuclei reticularis pontis oralis and caudalis, the superior, medial and lateral vestibular nuclei, as well as the nucleus prepositus hypoglossi, the abducens and the hypoglossal nucleus, the magnocellular reticular formation, the inferior olive as well as the pontine and medullary raphe.

754.1

AFFERENTS TO THE SIMPLEX LOBULE MICROZONE RELATED TO EYELID MOVEMENTS IN THE CAT. A. Gruart*, J.A. Armengol and J.M. Delgado-García. Lab. de Neurociencia, Fac. de Biología and Dpto. de Ciencias Morfológicas, Fac. de Medicina. Univ. de Sevilla, Sevilla, Spain.

The topographical location of brainstem and deep cerebellar neurons projecting to the eyelid microzone of the simplex lobule was studied in the cat. Under general anesthesia, horseradish peroxidase (Sigma type VI, 1.5-3 μL of a 30% saline solution) was injected in six adult cats in the area of the simplex lobule activated by the electrical stimulation of the supraorbitary branch of the trigeminal nerve. After 24-48 h of survival, animals were perfused and their brains processed according to the tetramethylbenzidine procedure. Deep cerebellar nuclei neurons projecting to the eyelid microzone were located in the dorsomedial aspect of the most rostral part of the posterior interpositus nucleus. Contralateral labeled brainstem neurons were located in the spinal trigeminal and prepositus hypoglossi nuclei, in the rostral medial accessory inferior olive, and in the lateral and dorsal subdivisions of the pontine gray. Ipsilateral projections arised from the lateral reticular, the external cuneate nuclei, and a discrete zone of the medial pontine gray. Bilaterally labeled neurons were placed within the lateral tegmental area, the paramedian reticular nucleus, and the reticularis tegmenti pontis nucleus. This complex pattern of afferents suggests that the simplex lobule plays a key role controlling the different types of lid movement.

754.2

ABSENCE OF AGE-RELATED CHANGES IN EYELID KINEMATICS. R.S. Baker*, J.C. Chuke, B.R. Rouholiman, M.W. Stava, and J.D. Porter. Depts. of Ophthalmol. & Anat. & Neurobiol., U. of Kentucky Med. Ctr., Lexington, KY.

Neurologic disorders that affect blinking (e.g., blepharospasm, Parkinson disease) often manifest at ≥50 years of age. Eyelid kinematics provide insight into the altered blink reflex excitability that may accompany disease. To evaluate the normal aging pattern, we used a modified scleral search coil technique to analyze blinks in normal human subjects for each decade between 40-90 years (n=5/decade). Mean amplitude and peak velocity decreased across this interval for both down (amp: 38.8° ± 4.4 to 26.0° ± 2.8, $p<0.05$; vel: 1200°/sec ± 162 to 818°/sec ± 131, $p<0.05$) and up (amp: 36.0° ± 5.3 to 24.2° ± 3.4, $p<0.05$; vel: 587°/sec ± 43 to 439° /sec ± 72.2, n.s.) phases of spontaneous blinks. Much of the decrease could be attributed to reduced palpebral fissure width (12.4 to 10.2 mm, $p<0.05$). Despite down phase amplitude reduction of >30%, down phase duration remained constant. Blink down phase main sequence (amplitude-peak velocity) slope is an indicator of reflex excitability. There was no change in main sequence slope across the age range tested, suggesting that reflex excitability was unchanged. Blink conjugacy also showed no change, with consistently high interocular correlations for amplitude/peak velocity across the intervals studied. Blink rate was unchanged across the age range tested. Together, these studies evaluated the hypothesis that blink excitability changes as a function of age, with the hyperexcitability that is seen in blepharospasm and Parkinsons representing extremes of normal aging. The peripheral eyelid and/or muscle changes leading to age-related ptosis were not accompanied by altered reflex excitability. These data then suggest that disease-related alterations in blink behavior and, by correlation, in the blink neural control systems, are not simply an exaggeration of normal aging changes. Supported by NIH EY010760, the Benign Essential Blepharospasm Research Foundation, and Research to Prevent Blindness.

754.3

ACTIVITY OF BRAINSTEM NEURONS DURING BLINK-SACCADE INTERACTIONS. L. E. Mays* and D. W. Morrisse. Dept. of Physiological Optics and Vision Science Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

Recently, we have shown that pontine omnipause neurons (OPNs) in the monkey pause for blinks as well as for saccades (Soc. Neurosci. p. 1404, 1993). Blink-related pauses last ≈ 140 ms and are easily elicited by air puffs directed at the face. Current models of saccade generation suggest that neuronal activity builds up in other saccade-related neurons prior to each saccade, and that turning off OPNs prematurely during this build-up period will result in slow saccades. We tested this hypothesis in three rhesus monkeys by timing the delivery of an air puff to produce a blink before, during, or after a saccade to a visual target.

The occurrence of a blink just before a saccade resulted in a slowed saccade, often with a complex velocity profile. Blinks which occurred >70 ms prior to the expected onset of the saccade had little or no effect on the saccade. Horizontal short-lead burst (SLB) neurons were recorded during blink-saccade interactions in two monkeys. SLB activity was reduced for those saccades that were slowed by blinks. This result indicates that the slowing of saccades by blinks is not due simply to co-contraction of extraocular muscles or other mechanical effects. Surprisingly, the activity of saccade-related burst neurons in the superior colliculus was also reduced for blink-slowed saccades. (Supported by NIH grants EY03463 and EY03039 and by the McKnight Endowment Fund for Neuroscience.)

754.4

EFFECT OF AGE ON BLINK REFLEX EXCITABILITY C. Evinger[1]*, E.J. Schicatano[1], A.K.E. Horn[2], J.J. Pellegrini[3]. [1]Dept. Neurobiol. & Behav, SUNY Stony Brook, Stony Brook, NY 11794-5230, [2]Inst. Neuropathol.,Univ. Munich, Munich, Germany, [3]Dept. Biol., College of St. Catherine, Minneapolis, MN 55105

Uncontrollable spasms of lid closure are most common in middle aged and elderly individuals. Increases in blink reflex excitability accompany the development of lid spasms. Nevertheless, the connection between increased blink reflex excitability, aging and the development of lid closure spasms is unclear. We investigated these associations in old and young guinea pigs (GPs).

Young (<6 months) and old (>2 years) GPs were chronically implanted with electrodes for bilateral stimulation of the supraorbital branch of the trigeminal nerve (SO) and recording of emg activity (OOemg) of the lid closing, orbicularis oculi (OO) muscle. We determined blink reflex excitability by presenting pairs of identical SO stimuli with interstimulus intervals (ISIs) of 50-1250 ms and compared the magnitude of the OOemg evoked by the 2nd stimulus to that evoked by the 1st stimulus. After determining normal blink reflex excitability, the auriculopalpebral branch of the VIIth nerve was crushed to weaken the OO muscle and induce compensatory increases in blink reflex excitability.

Blink excitability and the compensatory response to OO weakening differed between old and young GPs. In old GPs, the lengthened duration of the R2 component of the blink reflex promoted increased excitability of the blink reflex at short ISIs (<250 ms) over that of young GPs. Weakening of the OO muscle potentiated blink reflex excitability at short ISIs for both groups but the increase was larger and recovered more slowly in old GPs. Nevertheless, old GPs did not develop spasms of lid closure. Thus, blink excitability increases with age, but the development of lid spasms is not an inevitable sequelae of increased excitability. Supported by EY07391 (CE) and IT32NS07371 (EJS).

754.5

BLEPHAROSPASM PRODUCED BY A COMBINATION OF DOPAMINERGIC LESIONS AND ORBICULARIS OCULI WEAKENING. E.J. Schicatano* and C. Evinger, Depts. Neurobiology & Behavior and Ophthalmology, SUNY Stony Brook, Stony Brook, NY 11794-5230

Unilateral destruction of midbrain dopamine neurons dramatically increases the excitability of the blink reflex by reducing tonic inhibition of the trigeminal nucleus. Increases in blink reflex excitability also occur as a compensatory response to weakening of the orbicularis oculi muscle (OO). We investigated the relationship between these two mechanisms of blink reflex plasticity.

Rats were implanted with electrodes to stimulate the supraorbital branch of the trigeminal nerve (SO) bilaterally and record the OO activity (OOemg) chronically. Sensorimotor excitability was measured by presenting pairs of identical SO stimuli with interstimulus intervals of 50-300 ms and comparing the magnitude of the OOemg evoked by the second stimulus to that evoked by the first. Interactions between SO and auditory evoked blinks were also assessed. We tested blink reflex excitability in two groups of alert rats. In both groups, we removed a 2 mm section of the facial nerve innervating the OO. The second group received a small, unilateral 6-OHDA lesion (<30% cell loss) of midbrain dopamine neurons 20 days before the nerve transection.

Both 6-OHDA lesions and facial nerve lesions alone produced modest increases in blink reflex excitability. The combination of the 6-OHDA and facial nerve lesions, however, elicited a large increase in blink reflex excitability accompanied by lid closure spasms such as occur with blepharospasm. Interacting auditory and SO blinks revealed that the facial nerve lesion exerted its greatest effect on trigeminal evoked blinks. Thus, the data suggest that dopamine neuron loss potentiates the normal compensatory increase in trigeminal nucleus excitability induced by weakening of the OO muscle.

Supported by EY07391 (CE) and IT32NSO7371 (EJS).

754.6

PSYCHOPHYSIOLOGICAL STUDIES OF THE BLINK RELFLEX IN THE ALERT CAT. J.F. Lorden*, M.S. LeDoux, J. Smith, & A. Weir. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

As background for studies to examine the modification of the blink reflex by drugs or manipulation of central pathways, behavioral studies were conducted in seven alert, adult male cats. Under anesthesia, the animals received chronic stimulating electrodes over the supraorbital nerve and recording electrodes in orbicularis oculi muscle for electromyography (OOemg). With a stimulus duration of 0.1 msec and a stimulus magnitude of 1.5X threshold, measures of latency, duration, area, and maximum amplitude (MA) were obtained. The cat, like other mammals, shows both a short-latency (R1) and a long-latency (R2) component in the OOemg. Similar to humans, the mean (± SE) latency for R1 in cat was 10.8 ± .2 msec and for R2, 37.0 ± .1 msec. For both R1 and R2, MA and area measures were significantly correlated. Only area and MA of the R1 and R2 showed significant change as a function of stimulus intensity. In a paired stimulus paradigm in which the interstimulus interval (ISI) was varied from 100-1200 msec, ratios were constructed for the OOemg area and MA by dividing the test response by the conditioning response. In this paradigm, a signficant linear relationship was observed only between ISI and R2 MA. (Supported by the Dystonia Med. Res. Fdn.)

754.7

FUNCTION-SPECIFIC SUBDIVISIONS OF THE NUCLEUS OF EDINGER-WESTPHAL AS REVEALED BY TRANSNEURONAL TRANSPORT OF WGA.
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In most vertebrates, preganglionic neurons of the midbrain parasympathetic nucleus of Edinger-Westphal [EW] project to the ciliary ganglion. In pigeons, the postganglionic cells innervate the two intraocular muscles, i.e., the iris and the ciliary muscle, and the choroid vasculature. Previous anterograde pathway tracing (Gamlin et al. '84), lesion and immunohistochemical (Reiner et al. '91) studies have provided evidence that EW is organized into three subdivisions corresponding to the postganglionic targets. Retrograde transneuronal transport from the iris or ciliary muscle demonstrates the functional specificity of these subdivisions. WGA injections into the anterior chamber (iris) retrogradely and transneuronally label EW cells in the caudolateral portion of EW. Vitreous injections of WGA label only this same pupillary subdivision, suggesting that the ciliary muscle is not as accessible to WGA as is the iris. Injections into the ciliary muscle label these same "pupillary" cells as well as neurons in a more rostromedial portion of EW. The most ventromedial region of EW remains consistently unlabeled. Counts of transneuronally labeled cells agree with previous estimates of the numbers of pupillary and accommodative neurons, respectively, in EW. Confirming the anatomical results, microstimulation in caudolateral EW produces pupillary constriction; accommodative responses are evoked medially and rostrally. Supported by NEI grants EY04587 (JE), EY07391 (CE) and EY04742 (WH).

754.8

CENTRAL CORRELATES OF VISUAL ACCOMMODATION IN HUMANS MEASURED WITH $H_2{}^{15}O$ WATER AND PET

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The purpose of this work was to identify human neural circuits involved in the execution of voluntary positive accommodative eye-movements (VPA). Although accommodative eye-movements can be elicited volitionally, the brain mechanisms subserving higher cortical computations involved in visual accommodation remain elusive. Normalized regional tissue activity (NrTA) was measured using positron emission tomography (PET) and the $H_2{}^{15}O$ bolus technique in 7 healthy, right-handed volunteers with normal vision during three counterbalanced tasks requiring monocular viewing of a checkerboard through a lens introduced into the line of gaze during alternate 1.5 s intervals: (i) passive viewing through 0.00 diopters; (ii) avoiding volitional accommodation and permitting checkerboard blur through -5.00 diopters (i.e., continuous reflexive accommodation); and (iii) attempting to maintain focus through -5.00 diopters (i.e., continuos execution of large amplitude VPA in response to the induced blur). Saccadic eye-movements were suppressed through central fixation upon a cross and were confirmed absent by EOG. Intersubject image averaging of scan pairs showed activation of several structures during VPA: bilateral striate and extrastriate visual cortices; insula; superior and anterior temporal cortices; and cerebellar vermis. VPA recruits an extensive network of heteromodal and unimodal visual association cortices, both novel as well as convergent with previous findings in non-humans (Supported by Department of Veterans Affairs & the Swedish Crown).

754.9

LATENCY AND DYNAMICS OF PUPILLOCONSTRICTION DETERMINED BY MICROSTIMULATION OF THE EDINGER-WESTPHAL NUCLEUS AND OCULOMOTOR NERVE IN THE PRIMATE.
R.J. Clarke* and P.D.R. Gamlin. Dept. of Physiological Optics, University of Alabama at Birmingham, Birmingham, AL 35294.

We have been studying the neural control of the pupil in alert rhesus monkeys. To better determine the characteristic latency and dynamics of the pupil plant, we have studied the pupilloconstriction that results from electrical microstimulation of the Edinger-Westphal nucleus (EW) or the pupillomotor fibers of the oculomotor nerve (OMN).

Alert rhesus monkeys fixated a dim laser spot on a tangent screen while the pupil diameter of both eyes was measured under infrared lighting using ISCAN RK-406 pupillometry systems. A microelectrode was lowered under physiological guidance either to the EW or the OMN. Microstimulation was carried out over a wide range of parameters (100-1000 Hz; 10-100 μA; 3-100 ms).

In response to brief stimulus trains, the pupil constricted with a latency of 80-120 ms. The shorter latencies were associated with higher stimulation currents. Peak pupilloconstriction occurred approximately 300-500 ms after stimulation and showed an exponential return to baseline with a time constant of approximately 300-600 ms.

These characteristics indicate that the pupil plant acts as a low pass filter and explain the sluggishness of pupillary responses. The first-order models of the pupil plant that have been proposed do not adequately explain these results and a third-order model is required to provide a more complete description. (Supported by NIH EY09380 and P30 EY03039).

754.10

SINGLE-UNIT ACTIVITY RELATED TO THE NEAR-RESPONSE IN AREA 8 OF THE PRIMATE FRONTAL CORTEX.
P.D.R. Gamlin* and K. Yoon. Department of Physiological Optics, University of Alabama at Birmingham, Birmingham, AL 35294.

We have previously reported neurons related to the near-response in a region of the nucleus reticularis tegmenti pontis (Gamlin and Clarke, J. Neurophysiol., 1995) that is reported to receive input from area 8 of the frontal cortex. We have therefore begun to study neurons in area 8 during vergence and ocular accommodation.

Single-unit recording from area 8 of an alert, behaving rhesus monkey identified 15 neurons that showed activity specifically linked to the near-response. Most of these cells exhibited transient increases in their firing-rate during the near-response. Some cells also displayed a tonic firing-rate that increased as a function of increases in convergence and accommodation. Microstimulation (30-50μA; 30-50ms; 500Hz) at the site of some of these neurons elicited increases in accommodation and convergence of approximately 0.2-0.5 diopters and 0.2-0.5 degrees respectively. An injection of fluorescent latex beads at the location of the recording sites showed that they were located immediately posterior and dorsal to the principal sulcus.

The region of area 8 related to saccadic eye movements includes the anterior bank of the arcuate sulcus and the immediate prearcuate cortex. The region involved in smooth pursuit eye movements includes the fundus of the arcuate sulcus. These regions have been termed collectively the frontal eye field (FEF). The present study suggests that the FEF may be involved in all voluntary eye movements and that it may extend anteriorly to include a region of area 8 related to vergence and ocular accommodation. (Supported by NEI Grant EY07558 and P30 EY03039).

754.11

THE PRIMATE OLIVARY PRETECTAL NUCLEUS AND ITS ROLE IN THE PUPILLARY LIGHT REFLEX.
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It is generally presumed that an olivary pretectal (OPt) nucleus to Edinger-Westphal (EW) nucleus projection is the central relay in the pupillary light reflex pathway. However, the organization of the OPt nucleus and its projections have not been described in primates. The tracers biotinylated dextran amine (BDA), biocytin and WGA-HRP were used in *Macaca fascicularis* monkeys to investigate these points. BDA injected along the path between the OPt and the oculomotor nuclei homogeneously labelled neurons whose somata were generally located in the periphery of the ovoid-shaped OPt nucleus. Their highly branched dendrites were directed toward and filled much of the center of the nucleus. A combined antero- and retrograde tracing approach was utilized to reveal the relationship between the pretectal projections and the preganglionic motoneurons. Labelled preganglionic motoneurons were restricted to the ipsilateral EW nucleus and its anteromedian nucleus extension following WGA-HRP injections into the ciliary ganglion. Biocytin injections into the OPt nucleus of the same animal labelled pretectal terminals bilaterally in and around the EW nucleus. These labelled pretectal terminals were concentrated within the middle third of the EW nucleus in its rostrocaudal dimension, and were more dense medially. The area in which pretectal axon terminals were found immediately adjacent to labelled preganglionic motoneurons, suggesting a direct connection between these two, was quite restricted. Neither anterogradely labelled terminals, nor retrogradely labelled cells were observed in the contralateral OPt nucleus following the pretectal injections. In conclusion, this study indicates that the dendritic organization of OPt projection neurons is suitable for producing very large visual fields, in agreement with their reported physiology. The bilateral OPt-EW projection, along with the bilateral retinal-OPt projection, represents an anatomical substrate for the direct and consensual components of the pupillary light reflex. Finally, only a small portion of the EW nucleus may subserve this reflex in monkeys. Support: NEI grant EY09762 (PJM).

754.12

THE SIMIAN PUPILLARY LIGHT REFLEX AND THE RESPONSE PROPERTIES OF ITS PRETECTAL INTERNEURON.
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By means of a CCD imaging system, we measured the pupillary response to brief, full-field flashes of light in 4 monkeys. As in man, the flash causes the pupil to undergo a relatively rapid constriction followed by a 50%-slower dilation to its original size. The responses were variable but, on average, the constriction amplitude and peak velocity increased and the response latency decreased with light intensity. The pupillary response was consensual.

To pursue the neuronal substrate of the pupillary light reflex, we recorded unit responses in the pretectum of 2 monkeys to 1 sec light flashes. All of our neurons lay in a tight cluster in close proximity to the pretectal olivary nucleus (PON), a small structure believed to contain the interneurons of the pupillary light reflex. Most of our neurons (32/37) exhibited a burst in firing to the light flash after an average latency of 54 ms (SD=19) followed by a sustained discharge that was less than the burst rate. The sustained discharge continued for the duration of the flash and returned to the pre-burst firing at a fixed latency after the flash turned off. The frequency of the sustained discharge increased with light stimulus intensity. The close temporal correlation of the firing patterns of these neurons with the visual stimulus and not with the pupillary response suggest that movement per se does not influence their discharge. In the vicinity of these "burst-tonic"neruons, a small number of transient neurons discharged only bursts at light on and off. Finally, electrical stimulation at the locations of the burst-tonic cells often evoked pupillary constrictions at currents as low as 20μa.

Taken together, our data suggest that the pupillary light reflex in monkeys closely resembles that in humans and that, as in other species, the PON serves as the site of its interneurons in the pretectum. Supported by grants EY00745, RR00166 and a Visual Training Research Grant #EY 07031.

754.13

DESCENDING PROJECTIONS FROM THE CORTICAL ACCOMMODATION AREA TO THE BRAINSTEM IN THE CAT.

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Previous studies indicated that the lateral suprasylvian area (LS) is related to the control of lens accommodation in the cat. It is known that the LS projects to the pretectum (PT) and the superior colliculus (SC). We previously indicated that both of the rostral SC and the PT are involved in the control of lens accommodation. In this study, we investigated connections between the cortical accommodation area in the LS and the brainstem accommodation areas in the cat.

This study was conducted in 6 cats, weighing 2.5-3.5 kg. WGA-HRP was injected by glass micropipettes into the accommodation area of the SC or the PT where accommodative responses were elicited with the low intensity < 20 μA, and studied retrogradely labeled cells in the LS. Secondly, accommodative responses to stimulation of the LS were compared before and after injection of muscimol (GABA agonist) into the the rostral SC. Following WGA-HRP injections into the accommodation area in the rostral SC, labeled cells were found mainly in the lower part of the medial bank of the medial suprasylvian sulcus (MSS), where is comparable to the accommodation area of the LS. On the other hand, following injections into the accommodation area in the PT, labeled cells were found mainly in the upper part of the medial bank of the MSS, where is comparable to the pupillary constriction area. After injections of muscimol into the SC, accommodative responses evoked by stimulation of the cortex were almost abolished. These findings suggest that the accommodation area of the LS projects exclusively to the rostral SC.

754.14

ACCOMMODATION-RELATED AREAS IN THE BRAINSTEM OF THE CAT.

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We previously indicated that the superficial~intermediate layers of the rostral superior colliculus is involved in the control of lens accommodation in the cat (Sawa and Ohtsuka, *Vision Res.*, '94). This area is thought to project to the pretectum (PT) and the mesencephalic reticular formation (MRF), which may be related to the control of accommodation. In this study, we conducted systematic mapping with microstimulation of these areas monitoring accommodative responses by an infrared optometer in the cat. This study was conducted in 8 cats. Tungsten microelectrodes were introduced stereotaxically into the brainstem, and the midbrain area located rostral to the SC was stimulated systematically. Accommodative responses were monitored with an infrared optometer (Nidek, AR-1100). At the end of each experiment, electrolytic lesion was made at the lowest threshold site. After the experiments, the animals were deeply anesthetized with pentobarbital sodium and were perfused transcardially. Locations of the electrolytic lesions and electrode tracks were identified. Low threshold areas for evoking accommodation were located at the postero-lateral portion of the PT, where was almost comparable to the nucleus optic tract (NOT), the olivaly pretectal nucleus (OPN) and the posterior pretectal nucleus (PPN), the nucleus posterior commissure (NPC) and the medial portion of the MRF. The OPN and the MRF are also thought to be involved in the control of pupillo-constriction and vergence eye movements, respectively. Accommodation, pupillo-constriction and vergence are functionally linked with each other as the ocular near response.

754.15

INNERVATION OF THE SMOOTH MUSCLE OF THE EXTRAOCULAR RECTI PULLEYS IN HUMANS AND MONKEYS.

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The recti extraocular muscles pass through connective tissue pulleys, located at the level of posterior Tenon's fascia, that stabilize muscle paths and control muscle pulling direction. Structures supporting these pulleys include substantial smooth muscle (SM) bundles, suggesting possibile dynamic regulation of pulley function.

To investigate neural control of pulley SM, histo- and immunohistochemical studies were performed on rhesus monkey and human orbital tissues. Two weeks prior to paraformaldehyde perfusion, both superior cervical ganglia of a monkey were injected with the lectin anterograde tracer PHA-L. Immunoperoxidase staining with a monoclonal antibody against human SM alpha-actin was used to confirm pulley SM. Mono- and polyclonal antibodies were used to demonstrate immunoreactivity (IR) to tyrosine hydroxylase (TH), dopamine ß-hydroxylase (DBH), catechol-O-methyltransferase (COMT), myelin basic protein (MBP), PHA-L, neuronal nitric oxide synthase (NOS), and snyaptophysin. The NADPH diaphorase reaction was also used as a marker for NOS, and the acetylcholinesterase (AchE) reaction for Ach.

Rich innervation was found in human and monkey pulley SM. Numerous axons terminating in motor endplates within SM bundles had IR to TH, DBH, and PHA-L but not COMT. Smaller axons and motor endplates were also demonstrated in SM using NADPH diaphorase and AchE, as well as NOS IR. The pterygopalatine and to a lesser extent the ciliary ganglia but not Edinger-Westphal nucleus had cells with NOS IR. Although large orbital nerves had MBP IR, none was found in pulley SM.

The smooth muscle suspension of monkey and human recti pulleys has a sympathetic projection employing norepinephrine via the superior cervical ganglion, a nitroxidergic projection from the pterygopalatine ganglion or other sources, as well as a cholinergic parasympathetic projection. These multiple projections suggest the existence of both excitatory and inhibitory control of recti pulley SM potentially subserving a dynamic role in regulation of ocular motility.

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CONTROL OF POSTURE AND MOVEMENT: PREHENSION

755.1

SYNCHRONIZATION IN FINGER MOTOR UNITS DURING THE PRECISION GRIP IN MAN.

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Since the multi-muscular, multi-joint system of the hand is biomechanically overspecified, the question was raised whether short-term synchronization is a strategy to facilitate the control of motor units (MUs) within and between muscles.

Synchronization was assessed in the precision grip during production of isometric force on 3 consecutive levels (1,2,3 N). Up to 5 simultaneously recorded intramuscular EMG signals were decomposed into their constituent MU potentials yielding 93 MUs (1 to 4 MUs/muscle). The maximal firing rate in the force range investigated was 14 Hz. For the vast majority of the MUs, the firing rate correlated with the force exerted. The MU spike trains were cross-correlated at each force level. In total, 75 of 166 intermuscular and 54 of 69 intramuscular MU pairs showed synchronization at any one force level. In pairs where both MUs were located in the extrinsic or intrinsic muscles, synchronization was more likely and stronger than in mixed pairs. Furthermore, among MUs of intrinsic muscles, pairs between thumb and index finger muscles (33/66) were synchronized to a comparable degree as pairs between muscles moving only one digit (12/21), thus indicating the presence of functional synergies. An important finding was that synchronization is unstable: it occurred on all 3 force levels only in one intermuscular (1DI-1PI) and in four intramuscular pairs (2xAbPL, AdP, FPL). We checked factors that might influence the probability of synchronization. Synchronized MUs had smaller differences between their firing rate (≤ 2 Hz) and lower firing rates than non-synchronized ones. Synchronization occurred preferentially just after recruitment of both MUs or of the MU with higher threshold.

In conclusion, stable synchronization of MUs does not seem to be a prerequisite for the control of finger muscles during force production in the precision grip, thus confirming previous findings at the global EMG level.

755.2

MODIFICATION OF PREHENSION KINEMATICS WHEN AVOIDING AN OBSTACLE

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Previously we had shown that, by altering the initial grip posture, the aperture reorganized without influencing the wrist transport. The aim of this study was to determine whether modification of the wrist trajectory affects the kinematics of the aperture. To examine this issue, either of two different plexiglass obstacles (20x1.5x9 cm or 20x1.5x 11 cm) were placed between the hand starting position and object. The subjects were asked to reach over the obstacle and grasp a dowel (9 x 2cm). The results showed that obstacle avoidance significantly prolonged transport duration, deceleration time, time to peak velocity and peak deceleration. Also, the time to peak aperture and the time to the peak opening and closing velocities of grip were significantly longer compared to those of control. However, when expressed as a percentage of transport duration, no differences were found in any temporal parameter of the grip. Primarily these data show that the kinematics of both components were influenced by obstacle avoidance. The temporal aspects of the grip were adjusted to those of the transport and suggest some degree of co-dependence. These findings support a view that grip aperture adjustments are influenced by the temporal profile of the transport component.

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M.Saling on leave from Inst.Norm.Pathol.Physiol., Slov.Acad.Sc., Bratislava

755.3

CONTROL OF PRECISION GRIP FORCE DURING BEHAVIOR THAT MIMICS SMOOTH PURSUIT. K.J. Cole* and R.S. Johansson. Dept. of Exercise Science, The Univ. of Iowa, and Dept. of Physiology, Univ. of Umeå, Umeå, Sweden.

Ramp loads that pull a gripped handle away from the hand cause the grip force to smoothly track the load force after an initial grip force pulse triggered by the increasing load force. The tracking behavior in 9 subjects was studied to determine the control mechanisms governing pursuit-like tracking, and the saccade-like pulses in grip force that often occur during tracking. We examined the possibility that subjects learn the smooth tracking response from the load shape on previous trials. Ramp loads of 4 N/s followed blocks of trials that began as a ramp load but which accelerated or decelerated after 0.75 s. The grip force during ramp loading was affected little or not at all by the conditioning loads. Also, the grip force closely tracked various load waveforms (ramps, slowly accelerating or decelerating loads). We conclude that the control process for the pursuit-like behavior uses sensory information from loading over a short time interval. Smooth tracking most likely results from a feedforward controller that consecutively extrapolates the current waveform during short time intervals. It operates in addition to a mechanism that is sensitive to unexpected changes in load force rate, or otherwise decreasing grip/load force ratios. Loads that accelerate from an initial ramp often cause repetitive pulsatile grip responses lasting about 250 ms, beginning as soon as 80 ms after acceleration onset. We suggest that stable grasp on the object is maintained by one control mechanism that operates nearly continuously, and by one that responds to discrete mechanical events at the fingertip or finger.

755.4

DEVELOPMENTAL CHANGES IN PREHENSION FOR CHILDREN OF 2 TO 9 YEARS OLD. M.Paré[1] and C.Dugas[2]* 1- Centre de Recherche en Sciences Neurologiques, Université de Montréal, Canada, H3C 3J7; 2- Département des sciences de l'activité physiques, Université du Québec à Trois-Rivières, Canada, G9A 5H7.

Anticipatory control is required to produce skilled manipulation of grasped objects. Adults program the entire movement with a single force-rate pulse by anticipating the required grip and load force to lift the object (Johansson and Westling, 1988). Recently, Forssberg et al. (1991,1992) have demonstrated that children are not capable of doing this until the second year, and that this anticipatory mechanism continues to develop over several years. The goal of the study was to demonstrate that the emergence of an adult pattern of grip and load force coordination is correlated with a an adequate safety margin adapted to the weight of the object. Moreover, we also wanted to quantify the relationship between the grip force and the vertical acceleration of the object during maturation. The subject (N=36) had to lift an object which could vary in size and weight using a precision grip. The object was instrumented with a pressure gauge to measure grip force. The displacement of the arm and object was recorded with a Peak Performance system. The results demonstrate three distinct stages in the development of the precision grip, from to 2 to 5 years old, from 6 to 8 years old and beyond 9 years old. At 2 years of age, the peak acceleration was negatively correlated (r= -0.51) with the peak grip force during lifting. By 3 years of age, the relationship between the peak acceleration and the peak grip force during lifting became positively correlated (r=0.32) and continued to consolidate until 9 years of age. Also, starting at 6 the safety margin steadily decreases and reaches a stable value by 9 years of age. (Supported by NSERC and FCAR)

755.5

TASK REQUIREMENTS IN GRASPING: KINEMATICS AND FORCES FOR TRANSPORTING, DRAGGING AND SELECTING. C.L. MacKenzie*, E.D. Graham and C.I. Ivens, Human Motor Systems Lab., School of Kinesiology, Simon Fraser University, Burnaby, B.C. Canada V5A 1S6

Task requirements and object properties affect kinematics (Kunesch, Binkofski & Freund, 1989) and forces (Fearing, 1986; Westling & Johansson, 1990) in grasping and manipulation. We studied tasks that require object transport and manipulation like dragging and selecting in human-computer interaction.

Human adults grasped a dowel (155 g, force transducers) using pad opposition, and placed it on small or large targets, forward or backward. They transported the dowel in three conditions: "naturally", by "dragging" (squeezing during transport), and by "selecting" (squeezing on placement). 3D kinematics of the hand and dowel (OPTOTRAK 3D motion analysis system, 200 Hz), grip and load forces were measured throughout free motion, compliant motion, and loaded motion phases of the movement. Kinematics during free motion had lengthened deceleration phases for "drag" and "select" conditions, compared to "natural"; kinematics during free motion and loaded motion phases had lengthened deceleration for smaller targets, attenuated for "drag" and "select" conditions; grip and load forces changed predictably with tasks, validating that subjects were performing as instructed; load forces during compliant motion peaked earlier for forward than backward transport. Results support anticipatory planning of parameters for using an opposition space (MacKenzie & Iberall, 1995) in the earliest phases of movement.

755.6

THE ROLE OF THENAR MUSCLE ACTIVITY IN THE CONTROL OF INDEX FINGER FORCE. D.H. Laidlaw*, M. Bilodeau, and R.M. Enoka. Dept. Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, OH 44195

Although the control of isometric abduction force by first dorsal interosseous (FDI) has been well characterized, little is known about the contribution of thenar muscle activity to index finger force under these conditions. To address this issue, experiments were designed to examine thenar muscle activity during maximum voluntary contractions (MVCs) and submaximal constant-force contractions (5% and 20% MVC). Surface EMG signals were recorded from the FDI, adductor pollicis (AP) and flexor pollicis brevis (FPB) of 6 young subjects. With the hand positioned so that the palmar surface rested on a horizontal surface, the thumb was either extended by about 90 degrees in the same plane or abducted by about 90 degrees so that it was in a plane perpendicular to the palmar surface. With the thumb in the abducted position, the statistically significant findings were: (1) less FDI EMG during an MVC; (2) reduced EMG in AP and FPB relative to FDI during the MVC and constant-force tasks; (3) decreased MVC abduction force exerted by the index finger; and (4) no effect on the control of submaximal force (coefficient of variation). These results suggest that, while thenar muscles are biomechanically coupled with first dorsal interosseous, activity in these muscles is not related to the control of index finger abduction force under isometric conditions. However, FDI activation appears to be less than maximal with the thumb in the abducted position

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755.7

TIME-DEPENDENT EFFECTS OF ANTAGONIST MUSCLE VIBRATION ON THE PERCEPTION OF ISOMETRIC FINGER FORCE. H. Henningsen*, B. Ende-Henningsen, S.Knecht, A.M. Gordon. Depts. of Neurology and Neurophysiology, Univ. of Wisconsin, Madison, WI 53705; Dept. of Neurology, Univ. of Münster, Germany; Dept. of Physiology, University of Minnesota, Minneapolis, MN 55455

We examined the contribution of agonist and antagonist muscle spindles to the perception and control of isometric finger force. Nine subjects were trained to produce a force level of 50 ± 25 g with the index finger. Following training, either the agonist (flexor digitorum superficialis) or antagonist (extensor indicis) was vibrated at 100 Hz for 5 sec prior to the onset of force. Agonist vibration resulted in an overshoot of the trained force level, likely due to autogenic reflex facilitation. Surprisingly, antagonist vibration resulted in a similar overshoot of the trained force level.

To further examine the role of antagonist spindles in the control of isometric force, we then vibrated the antagonist at various time periods relative to the onset of force. The time periods included 5 sec vibration 1) again prior to the onset of force, 2) overlapping the onset of force, 3) initiated at the onset of force, 4) initiated during the dynamic force increase, and 5) initiated during the hold phase of contraction. Antagonist vibration caused the largest overshoot of force if it occurred prior to the onset of force. The overshoot diminished progressively as the vibration was initiated later, with no overshoot when the vibration was initiated during the hold phase. We propose that sensory information from antagonist muscle spindles contributes to an internal model of the initial state of the effector prior to the initiation of force. Thus, this information mainly is used for the feedforward control of isometric force.

755.8

ILLUSORY FINGER MOVEMENTS EVOKED BY ENSEMBLE CUTANEOUS INPUT FROM THE DORSUM OF THE HUMAN HAND. D.F.Collins* and A.Prochazka Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada, T6G 2S7.

Movement illusions evoked by tendon vibration have established an important muscle spindle contribution to human kinesthesia. The extent of any cutaneous contribution is unclear, though recordings from skin receptors on the hand dorsum show that they can provide detailed kinematic information (Edin & Abbs, *J. Neurophysiol.* 65(3):657-670, 1991). We have now tested the hypothesis that ensemble stimulation of these receptors results in illusions of movement. Two techniques were used on naive subjects. First, electrical stimuli were delivered through an electrode array (4-16 pairs) on the dorsum of the right hand and fingers. The low intensity (1-1.7x perceptual threshold) stimulus pulses (80 μsec) were frequency-modulated from 0-700 Hz, sinusoidally at 0.3 Hz. Illusory movements were evoked in 11/24 subjects (44%) as revealed by matched movements of the opposite hand. These typically involved perceived flexion of the metarpo-phalangeal (MCP) joint(s) during periods of increasing stimulus frequency. Secondly, mechanical stretch of the skin was used to provide a stimulus more specific to receptors excited during natural movements. Small loops of string or squares of adhesive tape were stuck to the skin on the dorsum of the hand and fingers. These were attached to elastic bands which permitted even application of skin stretch, bi-directionally, away from the MCP joint at approximately 0.3 Hz. Illusory movements were evoked in 10/18 subjects (56%). These typically involved perceived extension of the MCP joint during periods of skin stretch. Six subjects were re-tested in a more controlled experiment to reduce and measure any actual movements of the fingers caused by the skin stretch. Three subjects perceived movements when actual movements were immeasurably small. The results show that cutaneous input alone is sufficient for the perception of movement. However, this cutaneous contribution to human kinesthetic sensibility is likely smaller than that of muscle spindles. Supported by the AHFMR and the Canadian MRC.

755.9

TACTILE LOCALIZATION DURING DISCRETE AND CONTINUOUS MOVEMENTS OF DIFFERENT SPEEDS. P. Dassonville* & J. C. Nagode, Brain Sciences Center, Minneapolis VA Medical Center, and Department of Physiology, University of Minnesota Medical School, Minneapolis, MN 55417.

The perceived location of a tactile stimulus in 3-D space has been shown to rely on an internal representation of hand in space that inaccurately encodes the timing and velocity of arm movements (Dassonville et al., Soc. Neurosci. Abs., 1993, 1994). On average, subjects report a stimulus presented to the finger during a fast discrete movement of the arm to be at the spatial location occupied by the hand approximately 90 ms after stimulus onset. Furthermore, the pattern of localization errors from some subjects indicates an internal representation that underestimates the hand's actual velocity. In the present study, we have characterized this representation during discrete and continuous movements of different speeds.

In the discrete movement task, subjects were instructed to point to the spatial location of a tactile stimulus presented to the index finger during a previous visually evoked movement of the hand performed at one of three distinct speeds (average speed = 1.2, 0.6 or 0.4 m/s). Localization errors were somewhat smaller for stimuli presented during the slow movements. However, the pattern of errors was consistent with an internal representation that underestimates movement velocity over a wide range of movement speeds. In a separate task, stimuli were presented to the finger during continuous tracing movements (average speed = 1.0, 0.7 or 0.35 m/s) around a circular template (175 mm diameter) displayed on a video monitor. Using the arrow keys of a keyboard, the subjects moved a cursor to the perceived location of each stimulus. On average, stimuli were perceived to occupy the location of the hand 47 ms after stimulus onset, with significantly smaller temporal shifts for stimuli presented during the fast movements (14 ms) than for those presented during the slow (92 ms). Thus, the representation of hand in space appears to match better the time course of fast movements. (Sponsored by PHS NRSA #1 F32 NS09531 & ONR #N/N0014-92-J-1905)

755.10

SPECTRAL ANALYSIS OF CUTANEOUS MECHANORECEPTOR ENG ACTIVITY RECORDED FROM A DIGITAL NERVE IN MAN. P. J. Slot and R. R. Riso*, Center for Sensory-Motor Interaction (SMI), Dept. of Medical Informatics and Image Analysis, Aalborg University, Fredrik Bajersvej 7D, DK-9220 Aalborg, Denmark

Activity recorded from the cutaneous afferents in the skin of the fingers is being studied to develop control signals for FNS based grasp restoration neuroprostheses. Contact, loss of contact and slippage events between the fingers and a grasped object evoke robust neural discharges. The purpose of the present studies is to develop techniques to discern these various mechanical events based only on the recorded nerve signals. The ENG was obtained using a tripolar cuff implanted chronically around the common digital nerve subserving the lateral border of the index finger in a volunteer subject with spinal paralysis and C6 level function. A servo-controlled mechanical stimulator was used to indent the skin at predetermined rates or to produce sliding movements of a rectangular shaped, flat surface, contactor. The recorded ENG activity was rectified, bandpass filtered (1kHz-5kHz), sampled at 12.8kHz and digitally stored for offline analysis. Mechanical signals representing contactor perpendicular and tangential forces and positions were sampled at 400 Hz and also stored. The ENG data were analyzed for spectral components using a PC and Fast-Fourier algorithms from Matlab software. Results, thus far, indicate that the frequency spectrum associated with the neural afferent activity evoked by the loss of skin contact has a more discreet frequency spectral composition then the activity evoked during the initiation of skin contact. These findings are encouraging since the utility of whole nerve afferent recordings for the control of FNS neuroprostheses is dependent on our ability to distinguish the underlying mechanical events at the skin object interface purely by examining the mechanoreceptor afferent activity.

CONTROL OF POSTURE AND MOVEMENT: REACHING II

756.1

ARM MOVEMENT RELATED ACTIVITY IN THE SUPERIOR COLLICULUS OF THE MONKEY DURING DIFFERENT EYE POSITIONS V. Stuphorn, E. Bauswein, W.Werner*& K.-P. Hoffmann; Allgemeine Zoologie u. Neurobiologie, Ruhr-Universität Bochum, D-44780 Bochum

Recently, Werner (Eur. J. Neurosc., 5: 335-340) has shown, that the Superior Colliculus (SC) contains neurons which are active before and during arm movements. Thus the primate SC might be also a part of the arm control system. We wanted to see in which reference frame these neurons operate, and therefore investigated the influence of different eye position and retinotopic target location.

In the first task, the 'Saccade-Reach-Task' (SRT), the monkey had to perform first a saccade and then a reaching movement to the target. So it was presented on the fovea when the monkey made his arm movement. In the second task, the 'Fixation-Reach-Task' (FRT), the monkey had to hold central fixation while reaching to the target. In this condition it was represented in the peripheral retina although the position of the target with respect to the head was the same as in the respective SRT.

We found two different types of reach cells. The first one discharges with an equal pattern of activity during movements to the same visual target in both tasks. So the activity of this group of cells does not depend on the position of the target in retinal coordinates. Therefore a transformation of the target representation into a head- or body-centered reference frame must have taken place upstream of this cells.

The second, much smaller, group of reach neurons discharge only, if the target is in a certain position on the screen and the monkey has to perform the FRT. Two conditions are neccesary for the activation of this cell type: a specific sensory pattern, which is represented in retinal coordinates, and the arm reaching movement. Thus this neurons might be part of a sensory to motor transformation.

We conclude from our data, that the majority of reach related cells in the SC operate in a nonretinotopic coordinate system. The existence of different types of arm related actvity indicates, that part of the reference frame transformation takes place in the SC itself. (Supported by Neurovision and Mucom)

756.2

IMPAIRMENT OF VISUOMOTOR TRANSFORMATIONS IN ALZHEIMER'S DISEASE. M.F. Ghilardi*, M. Alberoni, S. Marelli, F. Fazio, C. Ghez, M. Franceschi. INB-CNR, HS Raffaele, Universita' di Milano, Italy; Ctr. Neurobiol. & Behav., Columbia Univ. NYS P I, New York, NY 10032

In planning movements to a visual target, the nervous system integrates multi-sensory information to make the spatial transformations needed to bring the hand to the desired location. Since spatial localization may be impaired in Alzheimer's disease, we ask whether trajectory planning is altered in this condition.

Six patients and age-matched controls moved a cursor to 12 radial targets on a horizontal digitizing tablet from midline and lateral initial hand positions without seeing their arm. Target and cursor positions were displayed on a vertical screen in front of, or lateral to the subject. With cursor feedback, paths of both patients and controls were uniformly distributed. When the cursor was blanked during movement, controls showed directional biases varying with initial position (Ghilardi et al., J. Neurophys.1995). Neither extent nor direction varied with screen location. However, in patients movement paths were biased towards the location of the screen where the targets were being displayed for all initial hand position. For example, when the initial hand position was in the midline, hand paths directed forward were hypermetric and curved towards the midsagittal plane when the screen was also in the midline; when the screen was to the right, movements were biased to the right. For initial positions to the right of midline, paths showed corresponding biases according to the screen location.

We conclude that Alzheimer's disease impairs the ability to transform the arbitrary spatial reference frame of the computer screen into the hand-centered coordinate system normally used for motor planning.

756.3

CEREBELLAR PATIENTS MAKE INITIAL DIRECTIONAL ERRORS CONSISTENT WITH IMPAIRED CONTROL OF LIMB DYNAMICS A.J. Bastian*& W.T. Thach, Dept of Anatomy, Program in Physical Therapy, The IWJ Inst. for Rehab. Research, Wash. U. Sch. of Med., St. Louis, Mo. 63110.

The purpose of this study was to determine whether cerebellar patients make errors in early phases of reaching consistent with our hypothesis that the cerebellum generates feedforward signals to adjust for interaction torques. This hypothesis suggests that cerebellar patients would 1) make systematic directional errors during the earliest phase of a reaching and 2) increase the magnitude of these errors during faster reaching (when increased interaction torques are generated). We also addressed whether any such errors could be due to abnormalities in relative timing patterns of muscle activity or scaling of magnitude and/or duration of muscle activity. We studied normal and cerebellar subjects reaching in a parasagittal plane under three conditions: a "slow- accurate" condition where seated subjects made a self-paced reach to touch a 1 cm target on a 4 cm ball, a"fast-accurate" condition where subjects moved as fast as possible and touched any part of the 4 cm ball, and a "fast" condition where subjects moved as fast as possible towards the target, but were not required to stop on the target. Shoulder, elbow, wrist, and finger kinematics were videotaped and analyzed. EMGs from the anterior deltoid (AD), posterior deltoid (PD), biceps (BI), and triceps (TRI) were recorded and analyzed. Inverse dynamics equations were used to estimate elbow and shoulder torques.

Preliminary data show that cerebellar patients made increased errors in the initial direction of movement compared with control subjects. As the reach velocity increased, these errors increased in a direction that is consistent with an inability to adjust for the interaction torques generated at the elbow. The EMG analysis showed that the onset of entire pattern of muscle activity was delayed but the relative timing of the two muscles active during early phases of movement (AD and BI) was normal. These findings support the idea that the cerebellum helps to initiate movement and sends predictive signals that correct for errors caused by interaction torques. We speculate that the cerebellum compensates for interaction torques in the early phases of the movement by scaling of amplitude and/or duration of muscle activity. (Supported by The Foundation for P. T. and NIH grant NS12777).

756.4

THE USE OF PROPRIOCEPTIVE INFORMATION IN POINTING DEPENDS ON AVAILABILITY OF VISUAL INFORMATION: EVIDENCE FROM A DEAFFERENTED PATIENT. Fookson, O.I.,* Berkinblit, M. B., Cole, J. and Poizner, H. Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102, Institute for Problems of Information Transmission, Russian Academy of Sciences, Moscow, 101447, Poole Hospital NHS Trust, Poole, England

Analysis of errors of healthy subjects pointing to remembered targets presented in 3D space in a darkened room has demonstrated that subjects are significantly more accurate when pointing with vision of the moving fingertip than when pointing without any visual feedback (Berkinblit at al., 1995). However, the role of proprioception of the moving arm under these two conditions is unclear. To uncover its role, we tested a subject with a severe large-fiber sensory neuropathy with complete loss of proprioception in the extremities and in the body below the neck. Without visual feedback, this subject's pointing accuracy was about half that of the control subjects. However, when vision of the moving fingertip was available, his 3D pointing accuracy was even better than that of control subjects. We conclude that proprioceptive information from the moving arm is necessary for accurate pointing without visual guidance. However, since this rehabilitated deafferented subject showed the same pointing accuracy as controls when visual information about the moving fingertip was available, proprioceptive information from the moving arm is not critical to accurate pointing when partial visual information is available. Thus, under this latter condition, vision is dominant over proprioception .

Although the accuracy of pointing in darkness was significantly different for control and deafferented subjects, the mean maximum speeds across all target locations were similar. Moreover, the same speed of pointing movements was observed for both groups of subjects when they had vision of the moving fingertip. However, the capacity to vary movement speed was not the same for control and deafferented subjects. When subjects were required to move with different speeds (fast and slow), the speed of pointing varied 3 fold for the deafferented subject, whereas, control subjects could change their speed 8-9 fold.

756.5

LEARNING NON-LINEAR VISUOMOTOR MAPPINGS. J. R. Flanagan*, A. K. Rao. Queen's University, Kingston, Canada and Teachers College, Columbia University, New York, U.S.A.

Reaching movements are characterised by roughly straight hand paths in Cartesian space. However, the issue remains whether this reflects constraints on perception or production. To address this issue, we examined two-joint planar reaching movements in which we manipulated the mapping between actual and visually perceived motion. In particular, we used a non-linear transformation such that straight hand paths in Cartesian space would result in curved paths in perceived space and vice versa. Under these conditions, subjects learned, after about 500 trials, to modify their movements so as to generate straight paths in perceived space. This suggests that reaching movements are planned in perceived coordinates.

In a second experiment, we tested whether and how learning this non-linear visuomotor transformation transfers to novel targets. Subjects first performed over 300 trials among 3 training targets and were then presented with novel targets. We observed strong transfer to novel targets located both within and outside the workspace defined by the training set. This indicates that subjects did not simply learn a set of transformations specific to the training targets. However, transfer was not perfect. This suggests that subjects did not develop an accurate "rule-based" representation of the visuomotor mapping. The implications of these findings for theories of trajectory formation and learning are considered.

756.6

TRANSFER OF PRISM ADAPTATION BETWEEN RAPID AND SLOW REACHING MOVEMENTS. S. Kitazawa*[1,2], T. Kimura[1] and T. Uka[1]. [1]Neurosci. Sect., Electrotechnical Lab., Tsukuba 305, Japan; [2]PRESTO, JRDC, Japan.

Accurate reaching towards a visual target is initially disturbed when the visual field is displaced by prisms, but gradually recovers. To clarify whether the mechanisms of improvement are common among reaching movements with different velocities, transfer of the adaptive improvement between rapid and slow reaching movements was studied.

Four human subjects were trained to reach rapidly (<300 msec) or slowly (~5 sec) at a target that appeared at a random location on a tangent screen (400 mm away). Vision of the target and the hand was always blocked during the movement by liquid-crystal shutters, and allowed exactly after the index finger touched the screen. One experiment consisted of 3 sets (set 1-3) of 30 trials. In set 1, the subject wore no prisms and made 15 rapid reaching movements followed by 15 slow, or 15 slow followed by 15 rapid. In set 2, the visual field was displaced to the right or to the left by prisms (15 diopter) and the subject was required to make reaching movements with the same velocity as in the latter half of set 1 throughout the 30 trials. Initially the subject misreached the target by about 60 mm in the direction of the visual displacement, and the errors decreased with trials down to the control level by the end of set 2. In set 3, the subjects wore no prisms and were required to reach with either velocity. The initial errors in set 3 were 51±19 mm (mean ± S.D., n=16) when the movements with the same velocity were required in both set 2 and 3. In contrast, the initial errors were only 17±16 mm (n=16) when the required velocity in set 3 was different from that in set 2. Analysis of variance revealed that the magnitude of the initial error in set 3 did not depend on the velocity in set 2 ($p=0.8$) but on whether the velocity classes in set 2 and 3 were the same or different ($p<0.0001$). These results indicate that the major part of the adaptive improvement in rapid or slow reaching movements does not transfer to the movements with the other velocity, and suggest that independent mechanisms are involved in the improvement of rapid and slow reaching movements.

756.7

MOTOR LEARNING, BIMANUAL TRANSFER AND ISOCHRONY IN A TRACKING TASK. J. Ilmberger* and E. Hendrich. Dept. Phys. Med., Ludwigs-Maximilians-University Munich, 81366 Munich, Germany.

One basic aspect in motor learning is the amount and type of feedback involved in the movement. Freund (1986) proposed two movement categories: type I movements are slow and under focal sensory control, whereas type II movements are fast, overlearned and under sensory field control. The transition from type I to type II movements can be considered as motor learning and may be described as a change in several parameters: reduction in movement time, reduction of the number of velocity changes during the movement and development of isochrony (decoupling of movement time and movement amplitude).

These parameters were measured in a tracking task in healthy subjects. Subjects had to track a figure consisting of straight lines with a pencil; data were recorded with a digitizing tablet. Two groups of subjects started with either the right or left hand. After twenty trials, they changed the performing hand for another twenty trials. An isochrony index was computed for each trial using the movement times for different parts of the figure. The results show a learning effect in the beginning hand and a learning transfer to the contralateral hand in all the parameters described above for both groups of subjects, although the transfer of isochrony was smaller from the left to right hand than vice versa.

The results are contrasted with data from single patients suffering from brain injury or peripheral impairment showing that the learning parameters and the learning transfer are changed in different ways in these patients.

Reference: Freund, H.-J. (1986): Time control of hand movements. In: Freund, H.-J., Büttner, U., Cohen, B. & Noth, J. (Hg.), Progress in brain research, Vol. 64, Amsterdam: Elsevier.

756.8

ADAPTIVE INTERNAL MODEL OF INTRINSIC COORDINATES TRANSFORMATION DURING LEARNING OF A REACHING TASK. H. Imamizu*, Y. Uno and M. Kawato ATR Human Information Processing Research Labs., Kyoto, Japan

Recent computational studies have proposed that the central nervous system acquires internal models of coordinates transformation between task-oriented extrinsic space and intrinsic space such as joint angles. To investigate acquisition of the internal model, we virtually minified (1/2) the elbow angle and magnified (5/4) the shoulder angle of human subjects while they were aiming at targets. A position marker was attached to the subject's hand and its current altered position was displayed as a cursor on a CRT screen. This linear transformation in joint angles (intrinsic coordinates) corresponds to a nonlinear one between the hand plane and the screen (extrinsic coordinates). We investigated whether the subjects learn this transformation as the former or the latter one. The aiming error when they learned the transformation of joint angles of one arm using the same arm (consistent condition) was compared to that when they learned the same transformation using the opposite arm (inconsistent condition). The error in the inconsistent condition was significantly larger (p <.002) than that in the consistent condition. Furthermore, the error in the consistent condition became small after they learned the transformation using the opposite arm in the same condition compared with that when they learned it for the first time (p <.0001). That is, intermanual transfer of the learning effect was found in the consistent condition but not in the inconsistent condition. Results suggest that the subjects learned the transformation as a linear one in intrinsic coordinates in the consistent condition and that the central nervous system adaptively represented the transformation including intrinsic coordinates in the control of arm movements.

756.9

SELECTIVITY OF ADAPTATION IN MOTOR LEARNING
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We studied the specificity of the adaptation to perturbations during pointing movements. We asked human subjects to execute point-to-point movements between targets arranged in a star-like fashion. The movements were planar and the subjects were coupled to a manipulandum which was used both to record kinematic data and to perturb the subjects. The perturbations, which were normal to the movement and proportional to the movement velocity, significantly altered the trajectories. We tested the subjects after the perturbing forces were discontinued and observed aftereffects as a mirror image of the perturbed trajectories.

We initially trained subjects to make movements to a subset of the targets while being perturbed, and then tested them on all the other targets without applying perturbations. To test for generalization of learning we observed the magnitude of the aftereffects in the unperturbed directions and found that the aftereffects decreased with distance from the trained trajectories.

As a further test of the adaptation, we trained the subjects to two different perturbation patterns, associating each of them to a particular arm configuration while keeping the end point trajectory the same. The subjects learned the tasks and showed aftereffects specific for the field related to the posture to which they had been exposed.

Taken together, these results indicate that the effects of motor adaptation tend to be local to the region where training has occurred. In addition, our experiments are compatible with adaptation being represented in intrinsic coordinates.

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756.10

GENERALIZATION OF ADAPTATION TO CORIOLIS FORCE PERTURBATIONS OF REACHING MOVEMENTS J.R. Lackner* and P. DiZio. Ashton Graybiel Spatial Orientation Laboratory and Volen Center for Complex Systems, Brandeis University, Waltham, MA 02254

Visually open-loop reaching movements made in a rotating room are initially deviated in the direction of Coriolis force perturbations, C_F, generated during movements; endpoint accuracy and straight line paths are restored within ~10 reaches. Mirror-image aftereffects occur when rotation stops, revealing motor reprogramming to balance the velocity-dependent, non-contacting, inertial C_F perturbations. Here, we studied in seven subjects already adapted to 10 rpm rotation the effects of intensifying C_F by increasing reaching velocity ~1.5 times. Renewed endpoint and curvature errors in the C_F direction were initially generated. Subjects adapted to the new reaching speed, at the same rate as to the original one. Mirror-image aftereffects did not occur when the original reaching speed was resumed. Thus, after being exposed during rotation to the C_F fields generated by just two different reaching velocities, a generalized adaptation occurred such that changing reaching speed again did not affect reaching trajectory or accuracy. Supported by NAG 9-515 and NAWC N61339-94-C-0062.

756.11

DELAYED VISUAL FEEDBACK IN SINGLE JOINT MOVEMENTS. J.D. Cooke*, K.K. Maitra and N. Virji-Babul. Faculty of Applied Health Sciences, University of Western Ontario, London, Canada N6G 1H1

As part of our study of the role of visual and kinesthetic information in coordination of multi-joint movement, we have studied the effect of introducing visual delay in the feedback of limb position information during single joint movements. We studied wrist (30 deg) or elbow (70 deg) movements in a visually guided planar, flexion/extension task while the position of other joint was fixed. EMGs were recorded from wrist and elbow flexor and extensors. We introduced time delays of 100, 200, 400 and 800 ms in the visual feedback to the subject of limb position information.

With visual delay no changes were observed in the kinematics or the EMG patterns of single joint elbow movements compared to no delay condition. In spite of the fact that the wrist position was fixed, clear phasic EMG activity occurred in both the wrist flexor or extensor muscles. In the case of single joint wrist movements, the wrist tended to move slower with short delays (200 ms) and faster with long delays (400, 800 ms) compared to the no delay conditions. These kinematic changes were reflected in the wrist EMG patterns. Clear phasic EMG activity occurred in the elbow flexor and extensor muscles in this condition where the elbow position was fixed.

The data suggest that the CNS can more readily compensate for altered visual input during elbow movements than during wrist movements. EMG activities in muscles acting about the non-moving joint may reflect learned programs related to compensation for or utilisation of reaction torques during multi-joint movements involving the elbow and wrist.

756.12

DELAYED VISUAL FEEDBACK IN A PLANAR TWO-JOINT MOVEMENT. K.K. Maitra*, J.D. Cooke and N. Virji-Babul. Faculty of Applied Health Sciences, University of Western Ontario, London, Canada N6G 1H1

In movements involving more than one limb segment, little is known of the relative importance of the visual and proprioceptive information related to movement about the different joints. In the present study we examined movement kinematics and kinetics and related EMGs in a planar, two joint (elbow/wrist) movement task. The task required accurate positioning about both the wrist and elbow. Movement amplitudes were 30 deg (wrist) and 70 deg (elbow). We inserted temporal delays (200, 400 and 800 ms) in the visual feedback information to the subject of the position of either or both limb segments.

Changes observed in elbow and wrist kinematics were graded with the delay. At smaller delays, movement velocity at both joints was reduced (compared to no delay conditions). As delay was increased, movement velocities increased as did agonist EMGs. At the wrist, this increase in agonist EMGs was not accompanied by change in the antagonists. Kinematic and EMG changes occurred independent of whether delay was applied to either or both limb segments. Movements around the elbow retained their accuracy despite changed movement speed whereas wrist movement under- or overshot the desired amplitude. With increasing delays reaction torques at the wrist increased slightly while muscle torques showed relatively large increases.

The data suggest that altering the normal relation between visual and proprioceptive information not only affects the kinematics of individual joints but also the coordination between them. It also suggests that when this relation is altered, movement about the proximal joint is preferentially preserved.

756.13

RELIANCE ON VISUAL FEEDBACK IN THE PERFORMANCE OF ARM AIMING MOVEMENTS FOR TWO DIFFERENT AGE GROUPS. R. D. Seidler, G. E. Stelmach, and H. L. Teulings*. Motor Control Lab, Arizona State University, Tempe, AZ 85287-0404.

It is well documented that the elderly exhibit longer movement times than the young when performing simple aiming movements. One possible contributing factor to this slowing is that the elderly rely more on visual feedback, requiring them to move more slowly in order to process visuo-motor information. Subjects made a horizontal aiming movement with a lever through the horizontal plane towards the body midline to a target located 30 cm from the home position. When subjects moved the lever, a cursor on a computer monitor directly in front of the subjects moved by a corresponding amount. The elderly subjects were affected more than the young by removal of visual information regarding arm position, reflected by a greater increase in absolute error. Following extensive training, the young subjects exhibited reduced endpoint error when visual feedback of arm position was withheld while the elderly did not. The kinematic results also suggest that the elderly place a greater reliance on visual control when performing simple aiming movements, and that in contrast to younger adults, this reliance is not reduced with practice.

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756.14

PRACTICE SCHEDULE AND MOTOR LEARNING: CHILDREN VERSUS ADULTS. G. Pinto Zipp * and A.M. Gentile. Teachers College, Columbia University, NY 10027; Seton Hall University, NJ.

When several variations of a novel motor task are learned concurrently, Barth et al (1994) found that blocked practice (successive trials on one variation before switching to another) yielded better retention and transfer than variable practice (random order of variations). It was proposed that stabilizing a topological framework of the movement (Bernstein, 1967) is required during initial learning. Blocked practice permitted such stabilization and advanced learning; variable practice did not. Prior reports of a random practice benefit (Magill and Hall, 1990) had used simple tasks requiring scaling of an available motor pattern: thus, subjects were beyond initial learning. IN the present study, 12 children (8-10 yrs) and 12 adults acquired a novel motor pattern (frisbee throw) under blocked or variable practice. Adults were expected to have positive transfer from prior throwing skills and benefit less than children from blocked practice. Acquisition (ACQ) involved throwing at 3 targets varying in distance using a blocked or variable schedule. Absolute error from the target was measured. Subjects were tested for retention (30-min & 2-days after ACQ) and transfer (different object or different distances). Blocked practice resulted in better retention for children and adults with no evidence of an Age x Practice interaction. Children showed a blocked practice benefit on one transfer condition; adults did not. Thus, only modest support was found that blocked practice would enhance learning more for children than adults. Rather, findings suggest that variable practice of similar movements during early acquisition produced interference, prevented stabilizing a movement framework and impaired learning for both age groups.

COGNITION XI

757.1

RELATIONSHIP BETWEEN CORTISOL AND VERBAL MEMORY IN YOUNG ADOLESCENTS. M.M. Prostak, M.S. Hansen, and B.B. Sherwin*. *Department of Psychology, McGill University, Montreal, PQ, Canada, H3A 1B1.*

As part of a longitudinal investigation of the behavioral and psychological effects of steroid hormones during puberty, the present study examined the relationship between salivary cortisol levels and verbal memory in a sample of healthy young-adolescent males (n=66) and females (n=69). Immediate and delayed verbal memory was assessed by means of a paragraph recall task analogous to the logical memory subtest of the Wechsler Memory Scale. Saliva samples were collected on strips of filter paper at the end of the test session and cortisol levels were determined by radioimmunoassay. Salivary cortisol levels and verbal memory scores were significantly higher for females than for males ($p<.05$). Cortisol levels were not significantly related to immediate paragraph recall scores in either sex. However, girls with higher cortisol levels performed significantly better on delayed paragraph recall and exhibited significantly less verbal memory score decay than did girls with lower cortisol levels ($p<.05$). These results suggest that moderate cortisol elevation may facilitate particular aspects of cognitive performance in female adolescents.

757.2

ASSESSMENT OF COGNITIVE DEVELOPMENT IN ADOLESCENTS BY MEANS OF NEUROPSYCHOLOGICAL TASKS. P.L. Davies* and J.D. Rose. Psychology Dept., Univ. of Wyoming, Laramie, WY 82071.

Research has shown that development of the frontal lobes of the brain continues through late adolescence, in contrast to the earlier maturation of other cortical regions. Six tasks associated with performance of parietal lobe functioning (visual closure, proprioception, graphesthesia, stick construction, relational questions, extrapersonal orientation) and 6 tasks associated with frontal lobe functioning (Road Map Direction Sense, Visual Verbal Test, Wisconsin Card Sorting Task, Stroop test, Wang's test, and personal orientation) were administered to 72 subjects, 36 females and 36 males. These subjects were classified into 3 stages of development: prepubertal, midpubertal, and postpubertal using two pubertal assessment scales. ANOVA with age as a covariate resulted in stage main effects for performance on 10 tasks, nine of which were associated with frontal lobe performance. This suggests that the tasks associated with frontal lobe functioning show more improvement with pubertal development than tasks associated with parietal lobe functioning. These findings demonstrated that neuropsychological tests which are typically used to assess cognitive effects of brain damage are extremely sensitive to developmental changes in normal cognitive function.

757.3

ACTIVATION OF ANTERIOR CINGULATE AND PREFRONTAL CORTEX IN CHILDREN DURING A RESPONSE INHIBITION TASK WITH FUNCTIONAL MAGNETIC RESONANCE IMAGING (FMRI).
B.J. Casey*, Trainor, R., Orendi, J., Giedd, J., Castellanos, X., Noll, D.C., Cohen, J.D., Haxby, J., Jezzard, P., and Rapoport, J.L. Psychiatry Depart., WPIC, UPMC, Pittsburgh, PA 15213.

As a follow-up to our first study of prefrontal cortex activation in children with fMRI (Casey, et al, 1995), we have recently collected data from 3 normal children (8 to 11 years) and 2 young adults on a go-no-go task. The task required the subject to respond to any letter but an X with 75% of the trials being targets or non Xs. We have observed significant behavioral deficits in performance on this task by children with Attention Deficit Hyperactivity Disorder. We hypothesized that this task would activate target brain regions of the prefrontal and limbic basal ganglia thalamocotical circuits assumed to be involved in inhibitory control processes. As predicted, all 5 subjects showed activation in the anterior cingulate and dorsolateral prefrontal regions and 3 of the 5 subjects showed activation in the medial orbital frontal cortex. Activation decreased as a function of time on task for both the anterior cingulate and dorsolateral prefrontal cortex. The location and magnitude of activation was similar for the children and adults.

Casey, B.J., et al, (in press). Activation of PFC in Children During a Working Memory Task with fMRI. *Neuroimage*.

757.4

CHILDREN'S VERSION OF THE ALTERNATIVE IMPAIRMENT INDEX: A PILOT STUDY. A. M. Horton, Jr.*. Psych Associates, Towson, MD 21204

Recently, the Alternative Impairment Index (AII) has been proposed as a new measure of neuropsychological impairment in adults. The AII is composed of scores derived from the Halstead-Reitan Neuropsychological Test Battery. This pilot study investigated the feasibility of a Children's Version of the AII. Test records for 16 children (4 Normal Controls, 3 Brain Tumors, 1 Brain Abscess, 1 Hemispherectomy, 1 Encephalitis, 4 Traumatic Brain Injury, 3 Learning Disabled, 1 Behavior Problems) between the ages of 9 and 14, who have been administered the complete Halstead-Reitan Neuropsychological Test Battery for Older Children, were obtained and the Children's Version of the AII and the Children's Total Neuropsychological Deficit Score were compared on agreement for level of severity. The results (i.e., 56% or 9/16 correct agreement) suggest weak levels of agreement.

757.5

MISMATCH NEGATIVITY INDICATES SPEECH DISCRIMINATION IN PRETERM INFANTS
M. Cheour-Luhtanen*[1], K. Alho[1], K. Sainio[2], K. Reinikainen[1], M. Pohjavuori[2], M. Renlund[2], O. Aaltonen[3], O. Eerola[3], and R. Näätänen[1]

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Recent studies have demonstrated auditory discrimination in infants. However, there is no direct evidence of auditory discrimination in the infants before the conceptional age of 40 weeks. Here we report first neurophysiological evidence that even preterm infants discriminate speech sounds.

Auditory event-related potentials (ERPs) were recorded to Finnish vowels /i/ and /y/, and to a boundary /y/i/ stimulus in preterm infants (n=11; conceptional age 30-35 weeks) with no major neurological findings. ERPs to the deviant vowel /i/ were significantly negatively displaced in relation to ERPs to the standard vowel /y/. ERPs to the boundary stimulus /y/i/ did not significantly differ from those to the standard vowel /y/.

The negativity to the deviant vowel /i/ resembled the auditory mismatch negativity (MMN), elicited in older children and in adults by change in a repetitive auditory stimulus (even when the sounds are unattended). Judging from the scalp distribution of the present negativity, this response seems to be the preterm equivalent of the adult MMN. Thus MMN might provide a new method for assessing the adequacy of central auditory processing in preterm infants.

757.6

LONG TERM CHANGES IN rCBF DURING COGNITION FOLLOWING SEVERE CLOSED HEAD INJURY: A PET STUDY. B.S. Kirkby*, J.D. Van Horn, Esposito, G., T.E. Goldberg, D.R. Weinberger, K.F. Berman. PET Unit, CBDB, NIMH, NIH, 10/4N-317, 10 Center Dr., Bethesda, MD

To assess functional reorganization following closed head injury (CHI), we measured regional cerebral blood flow (rCBF) in 11 unmedicated, severely-injured patients with good recovery (8 males; mean age=31; mean time post-injury=7.3 yrs, range=0.5-18 yrs) during the Wisconsin Card Sorting Test (WCST) and a sensorimotor control task (WCSTcon) using the oxygen-15 water positron emission tomography (PET) method. These subjects were compared to 11 normal controls matched for age, sex, education, and handedness. Absolute rCBF (ml/min/100g) was determined on a pixel-by-pixel basis with a least squares method. Regions of interest were individually drawn on the subjects' coregistered MRIs and applied to the rCBF data after pixel-by-pixel normalization to the global mean. Group differences in activation (WCST-WCSTcon) were analyzed using unpaired t-tests. No differences in mean global flow CBF values were found for either the WCST (CHI =48.2±11.7; controls=46.3±4.46) or WCSTcon (CHI=49.1±8.8; controls=46.6±7.4). Moreover, patients performed equally well as controls on all measures of the WCST. In contrast, patients showed lower activation in the anterior cingulate (p=0.01) but higher activation in the inferior portion of the left inferior frontal gyrus (LIFG) (Brodmann's 44, 45, 47) (p=0.03). In light of recent evidence for a role of the anterior cingulate in attentional processes, reduced activation in this region in patients might relate to difficulties in attention, a commonly reported deficit following CHI, that were not reflected in WCST performance. Increased activation, on the other hand, was unexpected given the predominance of damage to the frontal lobes and its connections following CHI. In the context of normal task performance by the patients, the "over recruitment" of this frontal area might represent an attempt at compensation for inefficiencies in other prefrontal or extraprefrontal areas during performance of this task.

757.7

PRE- AND POST-SURGICAL ASSESSMENT OF VISUAL DISCRIMINATION FOLLOWING HEMISPHERECTOMY
A. Schiavetto*[1], P. Poirier[2], Y. Lakmache[1], J.G. Villemure[3] and M. Lassonde[1]

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Studies of hemispherectomized patients have often reported the presence of residual visual abilities in the blind field of these patients. These results, however, have often been challenged. The present study aimed at further verifying the presence of such "blind-sight" by comparing the pre- and post-operative performance of a patient submitted to a variety of visual tasks. The patient, A.M., suffered from intractable epilepsy since childhood. She had her first seizure at the age of 2.5 years. An EEG carried-out at the time showed epileptic activity in the central temporo-parietal regions of the right hemisphere. This activity remained unchanged until her hemispherectomy at age 17 (1993). The first set of studies was aimed at evaluating the patient's ability to detect and localize stationary and moving visual targets. In the second set of experiments, color, form and letter discrimination was also assessed. In the localization experiment, A.M. was significantly less precise than controls in the left hemifield before surgery although she could detect the presence of the visual stimuli in that field. After surgery, she no longer detected any light stimulus in her left visual field. As pertains to color, form and letter discrimination, while A.M. consistently made errors in both hemifields before surgery, her performance improved postoperatively, her errors being confined to left hemifield presentations. In fact, A.M. performed at chance whenever visual stimuli were presented in her left field. Overall, these results show that, in a patient with longstanding hemispheric epileptogenic dysfunction, the removal of the deficient hemisphere results in an improvement of the visual abilities of the residual hemisphere. Our results, however, do not support previous findings of the "blindsight" phenomena after hemispherectomy.

757.8

EVENT-RELATED BRAIN POTENTIALS (ERPS) INDICATE THAT BLINDNESS WITH LATE ONSET AFFECTS AUDITORY PROCESSING IN HUMANS K. Alho*, T. Kujala, A. Lehtokoski, A. Leinonen, and R. Näätänen Cognitive Psychophysiology Research Unit, Department of Psychology, FIN-00014 University of Helsinki, Finland

Our previous results have shown that blindness with an onset during the first years of life affects the N2 component (peak latency about 200 ms from stimulus onset) of ERP associated with auditory discrimination, the N2 being more posteriorly distributed in early blind adults than in sighted controls. These results, supported by magnetoencephalographic source localization, suggest that in early blind humans, posterior cortical areas normally involved in vision would participate in auditory processing. In the present study, ERPs to repetitive 600 Hz standard tones and to 660 Hz deviant tones occurring infrequently among standard tones were recorded at 23 scalp sites from 8 sighted subjects (age 21-32 yrs) and from 16 blind subjects (age 21-35 yrs). In 8 of the blind, the blindness caused by a peripheral visual deficit had started at the age of 12 or later, and in the other 8, before the age of 2. When the subjects' task was to count the number of deviant tones in the tone sequence, the N2 to these deviant tones was in the early and late blind subjects distributed on the scalp posteriorly to the N2 of the sighted controls. This suggests that even blindness with a late onset may result in participation of visual brain areas in auditory processing.

757.9

EVENT RELATED POTENTIALS IN OBSESSIVE-COMPULSIVE DISORDER DURING VERBAL VERSUS NONVERBAL "ODDBALL" TASKS. **J.P. Towey, G.E. Bruder, C.E.Tenke, P.Leite, and M. Leibowitz, NYS Psychiatric Institute, NY**

Prior studies have found abnormal event-related potentials (ERPs) in patients with obsessive-compulsive disorder (OCD) compared to normal controls, including ERP evidence of left hemisphere dysfunction (Towey et al., 1993) and misallocated cognitive resources in OCD (Towey et al., 1994). The present study measured auditory ERPs of OCD patients and normal controls in both verbal (consonant-vowel) and nonverbal (complex tone) oddball tasks. Stimulus intensity, duration and repetition rates were matched to those in our prior study. ERPs were recorded from 4 midline sites (FzCzPzOz) and 13 lateral pairs. After correcting for eye artifact, ERPs were submitted to repeated measures ANOVAs using window averages for the following latency windows (ms): N100 (70-150), N200 (160-250), P300 (280-550) and Slow Wave (560-1000). Preliminary findings for 7 OCD patients and 8 normal controls for midline electrode sites were: (1) N100 amplitude. Whereas the control group produced larger N100 amplitudes to right ear than left ear stimuli; the patient group produced just the opposite asymmetry (Group x Ear, p<.05). This is consistent with prior findings of left hemisphere dysfunction in OCD. (2) P300 amplitude. P300 amplitude in OCD was significantly smaller during the nonverbal than verbal task over posterior scalp sites, but this task difference was not found for normal controls (Group X Task X Electrode: p<.01). (3) Slow Wave. OCD patients showed less positive slow wave than controls at central/parietal sites to targets (but not nontargets), which was evident frontally as greater negativity in OCD patients (Group X Condition, p<.05). OCD patients also showed greater Slow Wave to nontarget stimuli than did normal controls, supporting prior findings of misallocation of cognitive resources in OCD. **(Supported by MH44815)**

757.10

ASSOCIATION OF TEMPORAL PROCESSING AS MEASURED WITH AUDITORY ORDER THRESHOLD AND PERSONAL TEMPO IN PATIENTS WITH LESIONS OF THE CENTRAL NERVOUS SYSTEM. N.v. Steinbüchel, M. Wittmann and E. Pöppel.* Inst. Med. Psychol., 80336 Munich Univ., and Research Center (KFA), 52425 Jülich, Germany.

It has been demonstrated some time ago that patients with left hemisphere injuries show considerably prolonged temporal order thresholds (OT) which represent the time needed for event identification in a sequence. Instead of normal values of approx. 30 ms, lesions result in a prolongation of OT up to 100 ms or more. In a new study we replicated this finding and discovered in addition that the disturbance of temporal processing is limited to patients with aphasic speech problems. Patients with injuries of the left hemisphere without aphasic problems and patients with anterior or posterior right hemisphere lesions showed no such effects. Interestingly, a similar dissociation of functional disturbance is observed with a qualitatively different experimental paradigm. The subjects were asked to tap with their index finger a response key in a regular and convenient way ("personal tempo"). With this experiment the two following variables can be analyzed independently: 1. key touching time (KTT), i. e. the interval between the end of an agonistic and the beginning of an antagonistic finger movement, 2. pause interval (PI), i. e. the interval between successive KTTs. Whereas KTT appears to be closely linked to motor execution, PI appears to reflect a more central cognitive component, namely an individually chosen tempo of movement. For both these variables a similar result was observed as for OT. Only patients with aphasic problems showed temporal alterations (a significant prolongation of KTT and PI) whereas all the other patient groups compared to a healthy control group appeared to be unaffected. Thus, different domains of temporal processing (high frequency sensory decoding, motor execution, and volontary temporal control) appear to be closely associated. (Support was obtained by DFG and BMFT)

757.11

INFLUENCE OF ALCOHOL ON FRONTAL MIDLINE THETA ACTIVITY S. J. Laukka*, T. Järvilehto

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After the discovery of the relationship between the human EEG theta rhythm (4 - 7 Hz) on the frontal area and problem solving task (Arellano & Schwab, 1950) there have been studies indicating a positive correlation between theta activity and the level of performance. However, an increase of theta was reported also with alcohol that is usually associated with deterioration of performance. The occurrence of theta activity was studied in a simulated driving task. Two interdependent decisions had to be made at two crossroads guided by two traffic signs. The subject had to find the correct way to drive a car through a set of roads in a computer game. Feedback about quality of performance was given after each trial. Each trial lasted about 10 s with a pause of 2 s between trials. The EEG was recorded from Fz. Theta activity was analysed during seven consecutive sections (1610 ms) of the game. The results showed that successful behaviour produced more theta than unsuccessful behaviour. Alcohol significantly increased theta activity. Theta activity was enhanced after the second traffic sign and the difference between the correct and incorrect road selections disappeared. The results may be related to the general relaxing effect of alcohol as well as to the deterioration of the estimation of performance by alcohol.

757.12

CORTICAL DEFICITS OF SEROTONIN, 5HIAA, AND NOREPINEPHRINE IN ELDERLY DEMENTED SCHIZOPHRENICS: A POSTMORTEM STUDY Powchik P, Davidson M, Knott PJ*, Haroutunian V, Yang RK, McBorrough EM, Davis KL Dept. of Psychiatry Mt. Sinai Sch. of Med., NY, NY 10029

Background: Most persons with schizophrenia have cognitive impairment to some degree. In many elderly persons with schizophrenia, the impairment is severe enough to warrant an additional diagnosis of dementia. Since most cognitively impaired schizophrenics do not show evidence of common neuropathology nor do they show evidence of cholinergic deficits, we investigated whether cortical deficits of norepinephrine (NE), 3-methoxy-4-hydroxyphenolglycol (MHPG), serotonin (5HT), and 5-hydoxyindoleacetic acide (5HIAA) (deficits of which have been described in other dementing illnesses) occur in demented schizophrenics. *Methods:* Postmortem cortical brain tissue (Brodmann areas 8, 9, 24, 20, 21, and 17) derived from 19 elderly chronic schizophrenics who had antemortem cognitive assessments, 12 Alzheimer's disease cases, and 9 normal controls were examined for concentrations of NE, MHPG, 5HT, and 5HIAA using HPLC-EC. *Results:* Alzheimer's subjects showed marked deficits in 5HT, 5HIAA, and NE in all cortical regions as compared to controls. No differences in the entire group of schizophrenic subjects were found compared to controls in any brain region for any metabolite. However, compared to cognitively intacted schizophrenics (n=7), demented schizophrenics (n=12) showed marked deficits of 5HT, and 5HIAA in Brodmann areas 8, 20, and 21 and NE deficits in frontal cortex (all p's <0.001 by independent t-tests). There were no differences in age or history of neuroleptic exposure in demented and non-demented schziophrenics. *Implications:* This study demonstrates a potential cause of severe cognitive impairment in schizophrenia and a possible target for pharmacological remediation. The 5HT, 5HIAA, and NE deficits are consistent with previous findings regarding the role of these neurotransmitters in cognition and suggests that brainstem dysfunction may be present in demented schizophrenics.

757.13

PATTERN OF COGNITIVE DEFICITS IN SUBJECTS WITH PARKINSON'S DISEASE AND MULTIPLE SCLEROSIS. M. Best, R.F. White, R. Au, A. Cronin-Golomb*. Department of Psychology, Boston University, Boston MA 02215 and Department of Veterans Affairs Medical Center, Boston MA 02130.

The concept of "subcortical dementia" has been used to describe a pattern of cognitive impairments common to several disorders in which the initial or principal (though not exclusive) locus of pathology is one or more subcortical structures. The pattern comprises deficits in memory, visuospatial function, executive function, and motor skills. The question is raised as to whether subtle deficits in such capacities also may occur in subjects without obvious or generalized cognitive decline. We assessed cognition and motor performance in subjects with two "subcortical" disorders, Parkinson's disease (PD, n=19) and multiple sclerosis (MS, n=20). Both groups showed mild to moderate severity of motor signs on neurological examination. A normal control group was similar to the patient groups in age (mean=53 years) and education (mean=15.5 years). Tests were drawn from the computerized Neurobehavioral Evaluation System and included measures of psychomotor performance, attention, visuospatial function, executive function, language, and memory. The PD and MS groups were impaired on tests of psychomotor performance relative to the control group, and the MS group was additionally impaired on several measures of reaction time. The PD and MS groups differed in regard to which cognitive tests elicited performance levels below that of the control group. The differing patterns of performance raise issues concerning the concept of subcortical dementia.

757.14

METABOLIC AND ANATOMIC IMAGING OF THE MEDIAL TEMPORAL LOBES CAN LATERALIZE TEMPORAL LOBE EPILEPSY BUT COMMONLY-USED VERBAL AND NONVERBAL MEMORY TESTS CANNOT. **Z. Caramanos*, F. Cendes, M.A. Thomas, D.L. Arnold, and M. Petrides.**

Montreal Neurological Institute and McGill Univ., Montreal, Canada, H3A 2B4.

We compared, in 36 unoperated temporal lobe epilepsy (TLE) patients (25 left, 11 right), the abilities of commonly-used memory tests and quantitative neuroimaging techniques to lateralise the side of predominant seizure origin as determined by extensive clinical electroencephalographic (EEG) recordings. Patients' delayed recall of the Rey Auditory Verbal Learning Task, the Rey Complex Figure, and the Wechsler Memory Scale's Logical Memory, Associate Learning, and Visual Reproduction Tests was compared with their hippocampal volumes, as well as with their medial-TL levels of the neuronal marker N-acetylaspartate as determined by proton magnetic resonance spectroscopic imaging.

Although results on the neuroimaging measures were highly intercorrelated, they were not related to the patients' memory scores. Moreover, whereas patients with left and right TL EEG disturbances differed on their metabolic and anatomic measures of medial-TL integrity, they did not differ in their preoperative performance on the exmined measures of verbal and nonverbal delayed recall. Furthermore, whereas neuroimaging-based linear discriminant models were able to lateralize patients' EEG disturbance with 100% accuracy, models based on memory testing results were not much better than base rate information alone (*i.e.* 27 versus 25 correct).

These findings suggest that whereas these anatomic and metabolic imaging techniques are useful in the preoperative lateralization of TLE, these widely-used tests of verbal and nonverbal memory are not. Nevertheless, these and other neuropsychological measures provide important information regarding the patients' cognitive abilities and should be included in any thorough clinical evaluation of TLE.

757.15

REGIONAL CEREBRAL BLOOD FLOW DURING REPEATED EXPOSURE TO A VIGILANCE TASK IN ADULTS WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER. J.B. Schweitzer, T. Faber, C.D. Kilts, J.Votaw, J.M. Hoffman, and L.Tune*. Depts. of Psychiatry, Radiology, Neurology, Emory PET Center, Emory University, Atlanta, GA 30322.

There is increasing evidence that Attention Deficit Hyperactivity Disorder (ADHD) has a neurobiologic basis. Behaviorally these symptoms increase with repeated exposure to a task. Using the Paced Auditory Serial Addition Task (PASAT), an auditory vigilance task, we examined changes in regional Cerebral Blood Flow (rCBF) in 4 adult right-handed males with ADHD and 5 normal right-handed male controls. Subjects were scanned on two consecutive days with 2 resting state, 2 control and 2 PASAT conditions per day, each associated with [^{15}O]water administration. In this ongoing study, different task related increases and decreases in rCBF between the groups emerged. ADHD subjects showed significant activations in the primary visual cortex and visual association cortex during the PASAT compared to the control task ($p<.01$). Control subjects showed activations in the right parietal and left inferior frontal regions during PASAT ($p<.01$). Changes in rCBF with repeated task exposure, were assessed by subtracting images acquired on Day 1 during the PASAT from Day 2. In ADHD subjects there were increases in rCBF in the left, superior temporal lobe from Day 1to Day 2 ($p<.01$); however there were no significant decreases in rCBF from Day 1 to Day 2 ($p<.01$). Control subjects showed activations in the inferior frontal lobe, the superior parietal lobes, and the left gyrus rectus from Day 1 to Day 2 ($p<.01$). Control subjects also showed decreases in rCBF from Day 1 to Day 2 in the left temporal lobe and the cerebellum ($p<.01$). These results suggest that different brain regions, and perhaps behavioral strategies, are used by ADHD adults to perform vigilance tasks than adults without ADHD. Furthermore, the lack of decreases in rCBF seen in ADHD subjects with repeated PASAT exposure suggests a sustained processing demand, that is diminished with practice in control subjects.

757.16

SUPERIOR TEMPORAL GYRUS IN SCHIZOPHRENIA AND EPILEPSY. M.A.Eckert[a], E. Gautier[b], R. Gilmore[c], J.M. Kuldau[d], D. Bowers[c], T. Flynn[b], R. Quisling[a], & C.M. Leonard[b]. Depts. of Psychology[a], Neuroscience[b], Neurology[c], Psychiatry[d] & Radiology[a], Univ of FL, Gainesville, FL 32610.

Both schizophrenia and temporal lobe epilepsy (TLE) are thought to arise from temporal lobe damage early in development. MRI studies have demonstrated reduced superior temporal gyrus (STG) volume in schizophrenia, and reduced medial temporal lobe volume in epilepsy. To determine if reduced volume of the STG is specific to schizophrenia, we compared the left and right STG of TLE patients (13 left focus, 7 M and 6 F, mean age: 37.5 yrs; 9 right focus, 5 M and 4 F, mean age: 35.0 yrs) with that of schizophrenic patients (11 M and 3 F, mean age: 36.2 yrs). Volumetric analyses were performed on 3d MR images reformatted into 1mm coronal sections. The STG was measured between 5mm anterior and 5mm posterior to the anterior commissure (Barta, et al. 1991). There were no differences in the STG volume among any of the groups. There was a significant and positive relationship between *right* STG size and IQ in the schizophrenia patients. In contrast, in the *left* focus epilepsy patients, IQ was significantly related to the size of the *left* STG ($F=9.61$, $p<.05$). There was no relationship of IQ to the size of either STG in the right focus group. Differences in these brain-behavior relationships may be due to differences in the timing and location of early brain damage. Prenatal damage in schizophrenia may be early enough to allow, or massive enough to force, a shift of cognitive function to the right hemisphere. (Supported in part by the VA Medical Research Service and NS 93211).

757.17

SPARED FACE PROCESSING IN WILLIAMS SYNDROME: NEW PERSPECTIVES ON BRAIN-BEHAVIOR LINKS IN A GENETICALLY-BASED SYNDROME. M. Rossen* W. Jones, & U. Bellugi . Laboratory for Cognitive Neuroscience, The Salk Institute, La Jolla, CA 92037.

Williams syndrome (WMS) is a genetic syndrome of neurodevelopmental anomaly that involves mental retardation but presents a remarkable juxtaposition of impaired and intact mental capacities. Most prominently, linguistic functioning is preserved while problem solving ability and visual based cognition are impaired.

Good face processing ability is also characteristic of WMS, providing evidence of asymmetry in cognitive functioning within visual-based cognition. We report here on performance of WMS adolescents on three paradigms involving distinct aspects of face processing: Benton Face Recognition (Discrimination of unfamiliar faces); Warrington Recognition Memory for Faces (immediate recall of unfamiliar faces); and Mooney Face Classification (Perception of faces from unclosed contours). WMS subjects performed significantly better than age- and IQ-matched DNS controls on all three of these measures. Moreover, the WMS subjects were indistinguishable from normal chronological age-matched subjects on the Benton Faces task. Strong, significant inter-task associations exist among the face processing tasks; in contrast, no significant inter-task associations exist between the face processing tasks and WISC-R IQ, or between face processing and other visual-based processing tasks such as a non-faces closure task (KABC Gestalt Closure), or between face processing and indices of language ability such as comprehension of grammatical forms.

Brain morphology shows an intriguing link with face processing in WMS (Jones, Rossen, Hickok, Jernigan, & Bellugi, Neurosciences Abstracts, this issue). *In vivo* MRI data from nine WMS adolescents reveal a strong correlation between performance on Benton Faces and volume of gray matter in inferior posterior medial cortex, normalized by total supratentorial volume ($r=.89$, $p < .001$). Warrington Recognition Memory for Faces and Mooney Face Classification also correlate strongly with normalized IPMCG volume. These results are consistent with data from prosopagnosics on brain loci important for face processing and provide new data relevant to the neural systems underlying specific aspects of behavior.

757.18

BRAIN-BEHAVIOR LINKS: BRAIN MORPHOLOGICAL CORRELATES OF LANGUAGE, FACE, AND AUDITORY PROCESSING IN WILLIAMS SYNDROME W. Jones, M. Rossen, G. Hickok, T. Jernigan†, & U. Bellugi.* Lab For Cognitive Neuroscience, The Salk Institute, La Jolla, CA 92037. †Veterans Affairs Medical Center and Univ. of California at San Diego, La Jolla, CA, 92037.

Williams syndrome (WMS) is a genetic disorder involving an asymmetric profile of mental retardation, with relative sparing of language and face processing, extreme impairment in spatial cognition, and hyperacusis: an abnormal sensitivity to sound. Distinctive patterns of abnormal brain morphology in WMS have also been reported.

We report evidence of relationships between regional brain volumes and specific behavioral abilities in WMS. Using in vivo MRI data from 9 adolescents with WMS, brain regions were delineated by stereotactic parcellation of cortex into eight areas using three orthogonal planes. **Faces.** A strong correlation was found between performance on Benton Facial Recognition and volume of cortical gray matter in inferior posterior medial cortex (IPMCG) normalized by total supratentorial volume ($r=.89$, $p<.001$). IPMCG predominantly includes hippocampus, posterior parahippocampal gyrus and some retrosplenial cortex. Normalized IPMCG also correlated with two other tasks involving face processing: Warrington Recognition Memory for Faces ($r=.87$, $p<.01$) and Mooney Face Classification Test ($r=.72$, $p<.07$). None of the other seven cortical regions showed significant correlations with these three face processing measures. **Language.** A significant correlation was found between pooled performance on standardized language measures and a measure of volume of inferior frontal cerebrum normalized by total supratentorial volume ($r=.73$, $p<.025$). Other regions did not show significant correlations with language performance. **Hyperacusis.** Volume of Heschl's gyrus (A1), normalized by superior temporal gyrus volume, was examined in a separate study using three WMS subjects. An abnormally large A1 volume (relative to normals and matched Down syndrome subjects) was observed. These apparent WMS brain-behavior relations are consistent with existing evidence on localization of language, face, and auditory processing, and suggest that analyses in WMS may provide clues to the brain bases of behavior.

757.19

PRINT EXPOSURE IN DYSLEXIC ADULTS: EVIDENCE FOR AN INDEPENDENT PREDICTOR OF KNOWLEDGE. J.G. Foy*. Department of Psychology, Loyola Marymount University, Los Angeles CA 90045.

The clinical neuropsychological literature defines dyslexia as an impaired ability to read and comprehend written or printed words, but the etiological role of language deficits in this disorder has been a matter of considerable debate. Dyslexic children frequently have difficulties with phonological processing (e.g., Mann, 1993), but linguistic deficits are not always found in dyslexics (e.g., Aaron, Olsen, & Baker, 1985, Coltheart, 1983). Stanovich and his colleagues have shown that print exposure may be an independent predictor of knowledge in normal readers with varying degrees of reading comprehension ability (e.g., Stanovich & Cunningham, 1993). Since knowledge bases play an integral role in skilled reading (Kintsch, 1983), print exposure may be an important variable to control when studying populations with reading impairments. In order to investigate these issues, dyslexic adults (18-25 years) who had graduated from high school and were attending college were matched by age, gender, and cognitive ability with normal readers. Linguistic, cognitive and reading skills, including phonological awareness and print exposure, were tested in the subjects. Dyslexics did not differ in phonological skills from normal readers. However, dyslexics differed significantly from normal readers in print exposure. Multivariate statistical analyses revealed that print exposure was a significant predictor of knowledge, independent of cognitive ability. These results suggest that print exposure may play an important role in cognitive processing and should be controlled in studies of dyslexic subjects. Supported by a Loyola Marymount University Faculty Research Grant.

758.1

MATCHING PURSUIT ON ERP ANALYSIS J.J. Allen, B. Shen, J.G. Williams and J.B. Angevine* Dept. of Psychology, University of Arizona, Tucson, AZ 85721

Obtaining averaged event-related potentials (ERPs) requires many repeated presentations of stimuli or the use of single trial methods that are heuristic and thus imperfect. For single trial events, traditional analysis techniques (e.g., Fourier analysis) often fail to extract information from noisy signals reliably. By averaging a large number of trials, the signal-to-noise ratio of temporal patterns in ERPs may be enhanced, but at the price of losing frequency information. A recently developed technique, matching pursuit analysis, offers flexible decompositions of signal components localized in both time and frequency domains (Mallat and Zhang, 1993). We found that this technique could extract both temporal and frequency information from noisy single trial ERPs. Averaging as few as several ERPs over the transformed domain provided distinct temporal frequency patterns that corresponded to the classic P3 component of the ERP in a memory-assessment task. Random noise and systematic artifacts of ERPs could be rejected by simply subtracting appropriate time-frequency atoms. For us, this technique not only provided a more efficient and precise tool for analyzing averaged ERPs, but also suggested a new approach to the analysis of single trial events.

758.2

POSSIBLE FRONTAL LOBE CONTRIBUTION TO NEGATIVE REPETITION PRIMING S. Yamagata, S. Yamaguchi* and S. Kobayashi. Dep. of Internal Medicine III, Shimane Med. Univ., Izumo, 693, Japan.

Several lines of evidence have shown that the repetition priming effect is sensitive to the direction of attention. On the other hand, one recent study confirmed an inverse repetition effect to words at the unattended location. We studied the positive and negative repetition priming effects on attended and unattended words during a lexical decision task and hemispheric contributions on those effects using topographic analysis of event-related evoked potentials (ERPs). Subjects were asked to focus their attention to a word (vertically oriented Kanji letters) presented in one visual field and ignore the word in the opposite field during a task of detecting a non-word in the attended field. The field attended was informed by an arrow precue and word color. The identical word appeared sequentially 56 times at the attended and unattended visual field respectively at random. A positive ERP deflection to repeated words developed when the sequential words were presented in the attended location. This repetition effect was greater over the posterior scalp sites contralateral to the attended field. Non-attended repeated words generated a negative-going shift over the left frontal scalp sites regardless of the field of word presentation. These results suggest that the frontal lobe may contribute to the active inhibition of implicit word processing within the unattended channel.

758.3

FUNCTIONAL TOPOGRAPHY DURING PROCEDURAL LEARNING STUDIED WITH EVENT-RELATED DESYNCHRONIZATION MAPPING (PRELIMINARY FINDING) P. Zhuang, C. Toro, J. Grafman, P. Manganotti, L. Leocani, M.-P Deiber, J.Walters*, M. Hallett, National Institute of Health, NINDS, Human Motor Control Section, Medical Neurology Branch, Bethesda, MD 20892, USA.

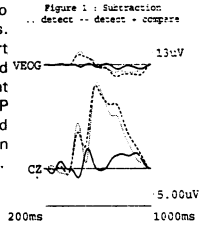
To explore the role of the human motor cortex associated with the development of implicit and explicit knowledge, we studied event-related desynchronization (ERD) as an indicator of localized brain activation during a visual motor task. EEG-signals were recorded from 3 right-handed subjects as they performed a variation of the serial reaction time task (SRTT). ERD was calculated within the alpha (8-12 Hz) band from EEG recording of 29 scalp locations from -1 to 1 sec around to the correct keypresses. During the data collection, all subjects developed implicit knowledge of the test sequence, which was reflected by diminishing response time and generation of explicit knowledge. ERD maps revealed localized alpha power reactivity overlying the contralateral sensorimotor hand area. The area of power reactivity became progressively larger until explicit knowledge was reached, after which the power spectra showed a declining power. These electrophysiological findings are in support of our previous results demonstrating the rapid functional plasticity of cortical motor outputs associated with procedural learning and with transfer of knowledge from an implicit to explicit state.

758.4

EVENT-RELATED POTENTIAL (ERP) CORRELATES OF TARGET DETECTION: EFFECTS OF TASK DIFFERENCES. I.Kiss* and A.W.Fazikas. Toupin Psychophysiology Lab, Northern Alberta Regional Geriatric Program and Division of Neuroscience, Univ. of Alberta, Edmonton, Alberta.

Ten subjects completed two visual "oddball" tasks involving identical stimuli; random length series of 3 to 9 individually presented digits. Target sets (3 digits or 3 item letter-digit sets) were presented between series in the *detect* condition. Subjects were asked to selectively respond to the 3 digit sets. In the *detect + compare* condition, subjects selectively responded to 3 number targets that matched the last 3 individual numbers presented. Error rates were similar across conditions.

ERPs elicited by target stimuli recorded from 4 midline scalp sites (Fz, Cz, Pz, Oz) included two major positive-going peaks (approximately 200 and 375 msec latency) in both conditions (Fig.1). Analyses of variance failed to support main effects of condition upon latencies. The effect of condition on amplitude just reached significance ($p = 0.049$) for the earlier but not the later peak (P300). This lack of amplitude and latency differences of the P300-like peak suggest insensitivity to considerable differences in task requirements. P300 may reflect the time course and effort involved in more general processes that proceed in parallel with, but independent of, divergent processes in distinct tasks. Digital ERP subtraction *[detect-(detect + compare)]* revealed ERP features (Fig.1) associable with comparison of working memory contents to external stimuli.



758.5

HEBBIAN INDUCTION OF AUDITORY CORTICAL RECEPTIVE FIELD PLASTICITY: EFFECT OF NUMBER OF TRIALS & CORTICAL STATE

Scott J. Cruikshank* and Norman M. Weinberger, CNLM and Dept. Psychobiology, UC Irvine, CA

Receptive field (RF) plasticity in the adult auditory cortex (Aud Cx) has been observed following learning (Weinberger et. al., 1990, *CINS*). We are investigating induction mechanisms of such plasticity, and have focused on Hebbian rules, because they have been implicated previously in neocortex (e.g., Shulz & Fregnac, 1992, *J Neurosci*; Ahissar et al, 1992, *Sci*). However, a preliminary report from our lab indicated that for most cells in Aud Cx, a "Hebbian" treatment (below) was insufficient to induce RF plasticity (*Soc Neuro Abs*, 1993). Since that report, we've investigated several factors that seem to affect the probability of observing RF plasticity following a "Hebbian" treatment. These include number of pairing trials and cortical EEG state.

Procedure: In Urethane anesthetized Guinea pigs, first, a single postsynaptic (postsyn) Aud Cx cell was isolated with a KCl-filled juxtacellular micropipette. During a baseline period, two Aud stimuli of differing frequency were presented, to activate different sets of presynaptic afferents for the recorded cell. The response of the postsyn cell to these two stimuli were recorded, to assess their synaptic strength. Next a "Hebbian" treatment was imposed in which one of the Aud stimuli (CS+) was paired with excitatory current to the postsyn cell, to enhance the pre/postsyn covariance. The other Aud stimulus (CS-) was paired with inhibitory postsyn current. After 60 pairings, the synaptic strengths were re-determined. For most cells another block of 60 pairings was imposed, to test the effect of number of trials on plasticity. EEG was also recorded.

Results: We calculated the relative response of the postsyn cell to the two Aud stimuli (CS+)/[(CS+)+(CS-)] for each trial in each period. The distributions of these relative scores were compared between periods (t-test, $p<.05$). All cells (n=28) were significantly controlled *during* the treatments. Only 3/28 (=11%) cells had significant relative increases for the CS+ *after the first* treatment block compared to baseline. However, there were much larger effects *after the 2nd* treatment block, with 7/22 (=32%) cells showing significant relative increases for the CS+. No cells had significant decreases. In addition, the probability of significant effects was greatest if the cortical EEG was "activated" during the treatment: only 2/13 cells that underwent treatment during EEG synchrony were affected, while 5/9 that underwent treatment during EEG activation (i.e., desynchrony or shift between synchrony and desynchrony) were affected. Other factors that might affect the probability of observing RF plasticity following a Hebbian treatment are being investigated.

NIMH #MH10432-03, NIDCD # DC02346

758.6

THE P3-LIKE LONG-LATENCY COMPONENT IN RATS: IS IT A HIPPOCAMPAL THETA WAVE? J. Brankack* and T. Seidenbecher, Inst. of Physiology II, Heinrich-Heine-University, Düsseldorf and Institut of Neurobiology, Magdeburg, Germany

Long-latency components of event-related potentials (P3 or P300) correlate with the ability of subjects to detect and process unexpected, novel or task relevant events. P3-like components were recorded in the neocortex and hippocampus of rats performing an auditory discrimination task, similar to the oddball paradigm used in human psychophysiological experiments. In anaesthetized rats surface and depth electrodes were implanted at several neocortical regions and the hippocampus. After recovery from surgery rats were trained to discriminate two auditory signals, a frequent irrelevant tone (T1) and a rare tone (T2) related to water reward. In response to T2 but not T1, P3-like components with a mean latency of 274 ms (range: 244 to 305) and a mean amplitude of 67 μV (range: 24 to 158) were recorded from the surface of the neocortex. The largest amplitudes were found in the anterior part of area Oc2MM situated above the hippocampal CA1 region. Powerspectra of differences between responses to T2 and T1 revealed peaks in the theta range (4-12 Hz) maximal at area Oc2MM. The amplitude increased with depth to a maximum of 298 μV in stratum oriens of the CA1. A polarity reversal occured at the pyramidal cell layer (-175 μV). The largest negative amplitude was found in stratum radiatum (-304 μV). It is suggested that the P3-like component in rats corresponds to a theta wave out of a burst of hippocampal theta cycles. This work was supported by the Deutsche Forschungsgemeinschaft grant Br1289/1-1.

758.7

N400-LIKE EVOKED POTENTIALS IN THE MACAQUE HIPPOCAMPUS

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Several animal models of the P300 exist,but non-human correlates of cognitive EPs of the N400-family have received scant attention. We recorded intracranial VEPs (peri-inial skull screw reference) from stereotaxic probes implanted in an adult female bonnet monkey (*M. radiata*). The task required her to distinguish familiar from unfamiliar human faces. Major components from a right hippocampal contact (coordinates A12.1,R11,D27: atlas of Szabo & Cowan,verified by MRI) were: Positive at 112±2.6 ms to peak,N156(12.9), N194(5.3),N266(14.0). Here amplitudes (μV) of the two largest components in the interior hippocampus *vs.* values at immediately superior and inferior sites:

Contact	N194		N266	
superficial (D25)	+ 90.7 ± 10.8	p<0.05	- 32.3 ± 18.4	p<0.05
interior (D27)	-110.6 ± 74.9		-115.5 ± 19.4	
deep (D29)	+ 78.7 ± 15.3	p<0.05	- 23.7 ± 24.6	p<0.05

The inversion of the N194 and the steep voltage gradients of both components suggest that the two are generated locally in the hippocampus. Thus large focal negativities occur in the monkey hippocampus in response to the same stimuli and in the same task that evoke large focal N310 and N430 components in the human hippocampus. While precise component homologies will require further work with additional tasks and in other subjects,monkey components appear generally to be earlier than in humans.

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758.8

NEURONAL ACTIVITIES IN MEDIAL PREMOTOR AREA OF MONKEY DURING LEARNING OF SEQUENTIAL MOVEMENTS.

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To investigate where and how the memory for sequential movements might be represented in the brain, we trained a Japanese monkey to perform a sequential button press task, "2x5 task". On pressing a home key, two of 16 (4 x 4) LED buttons (called 'set') were illuminated simultaneously. The monkey had to press them in a predetermined order which he had to find out by trial-and-error. A total of 5 sets ('hyperset') was presented in a fixed order for completion of a trial. A hyperset was repeated as a block of experiment until 20 successful trials were performed. The monkey experienced 965 newly generated hypersets. Short-term and sequence-selective learning occurred by repeating a particular new hyperset during a block of experiment. 14 hypersets were assigned to 'learned hypersets' which had been learned daily so that the animal could perform them with few errors.

We recorded spike activities of 154 single neurons in the medial premotor area as the monkey was performing 'learned' or 'new' hypersets. 68 cells (44%) showed differential activities between learned hypersets and new hypersets. Among them, 51 cells showed learning dependent changes within a block of experiment during which the monkey repeated a new hyperset. (1) 30 cells initially did not show task-related activities, but as learning proceeded, activities appeared that were related to hand movements of particular transitions or combinations of sequences. (2) 11 cells showed strong activities for new hypersets which became weaker as learning took place. These changes of activation pattern occurred for each new sequence repeatedly. These results suggest that medial premotor area participates in short-term and long-term learning of sequential movements.

758.9

REWARD-DEPENDENT ACTIVITY OF "DIFFERENTIAL DELAY NEURONS" IN THE PRIMATE PREFRONTAL CORTEX.

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In the delayed response task (DR), "Differential Delay Neurons" (DDN) are observed in the primate prefrontal cortex (PFC), which show differential activity between right and left trials during the delay period. They are considered to be related to the spatial working memory. In the last year's meeting, we reported reward-dependent delay-related activity in the PFC neurons. Here we report the activity of DDN in various reward situations.

Three monkeys were trained on the DR using several kinds of food and liquid reward (usually using the same reward for a block of 30 – 50 trials). On the 'Indirect method situation' position of a red light, and on the 'Direct method situation' position of reward itself (food reward only), indicated the correct side.

Many DDN were found to behave in different ways between Direct and Indirect and/or between food and liquid reward and/or among different food (or liquid) reward situations, showing different patterns of spatial specificity during the delay period. Among them there were such DDN which showed the spatial specificity either on Direct or on Indirect method situation, or either on liquid or on food reward situation.

The results indicate that DDN could be involved in retaining the difference of reward situation during the delay period besides being related to retaining spatial information and that the single PFC neuron could be involved in retaining more than one kind of information at the same time.

758.10

DISSOCIATION OF MOVEMENT RELATED NEURONAL ACTIVITY IN POSTERIOR CINGULATE CORTEX IN MONKEYS PERFORMING OCULOMOTOR AND MANUAL DELAYED RESPONSE TASKS.

S. Carlson*, P. Goldman-Rakic. Sect. Neurobiol., Sch. of Med., Yale Univ..

Single neurons in the posterior cingulate cortex (pCC) exhibit nonspecific delay period activity in monkeys doing oculomotor delayed response task (Carlson et al. 1993). In the present study we recorded single neuronal activity in the posterior cingulate cortex in monkeys performing both oculomotor and manual delayed response tasks (ODR and MDR resp.). The aim was to determine whether single neurons show task related activity also in the MDR task and whether the same or different neurons are engaged in the oculomotor and skeletomotor movements. As in the ODR task delay period activity was recorded also during the MDR task, and was not spatially tuned. The pCC neurons were "omnidirectional", i.e. active in the delay for all taget directions. Movement related neurons fired either during eye- or hand movements in pCC and were broadly tuned. The results indicate that neurons in pCC are nonspecific with respect to spatial mnemonic information; rather, their activity is related to preparation and execution of movements.

758.11

FUNCTIONAL ORGANIZATION OF THE ANTERIOR CINGULATE CORTEX IN MONKEYS.

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It has been suggested that the anterior cingulate (AC) cortex is involved not only in learning, but also in behavioral manifestation. In the present study, single neuron activity was recorded from the monkey AC during operant behavior based on discrimination of rewarding, aversive, and neutral objects. Of 303 neurons recorded from the AC, 63 responded in one or more phases of the task. Of these 63, 23 responded to visual stimuli (differentiating, 19; nondifferentiating, 7). These 19 responded to rewarding (5), aversive (6), or rewarding and aversive but not to neutral objects (8). Responses of 5 neurons that responded to aversive objects were readily suppressed by associating the aversive objects with reward (reversal). Of 15 neurons that responded mainly in the bar press phase, 5 differentiated bar pressing to avoid shock from bar pressing to obtain reward. Responses of 4 neurons were anticipatory, with gradually increasing activity between the start tone and a visual stimulus (object). There were topographic distributions of these responsive neurons in the AC; differential vision-related, differential bar press-related, and nondifferential bar press-related neurons were located from the anterior to posterior portion of the AC, respectively. The results suggest that sensory information is processed and transformed to motor command from the anterior to the posterior portions of the AC.

758.12

PARIETAL UNIT RESPONSES IN A CROSS-MODAL (VISUO-HAPTIC) DELAY TASK.

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The purpose of this research is to explore the reactivity of somatosensory cortical cells to visual stimuli and their role in the mnemonic retention of those stimuli for a prospective haptic choice. Monkeys were trained to perform a visuo-haptic task with a forced delay between a visual stimulus and a tactile choice appropriate to it. Each trial consisted of the following: (1) 3-sec visual *icon*--i.e., black and white pattern of vertical or horizontal stripes; (2) delay of 10-20 sec; (3) click signalling accessibility of two parallel rods, one with horizontal and the other with vertical ridges on the surface; (4) tactile comparison of the two rods; (5) choice (pull) of rod with ridges in the same direction as the stripes of the icon. Correct choice was rewarded with liquid reinforcement. Icon and relative rod position were changed at random from trial to trial. Units in cortical area S1 showed excitatory responses to the icon initiating the trial. The latency of the responses was about 200 ms. In some units ("memory cells"), the excitatory response persisted during the delay (retention period) in the form of sustained elevated firing. Some cells reacted differentially to the icons, i.e., more to stripes in one direction than in the other, and direction-differential firing continued during the subsequent memory period. In some instances, the preferred visual directionality of a cell was reflected by comparable preferred directionality in touch. These findings suggest that somatosensory units are accessible to visual information behaviorally associated with tactile information. Further, the results suggest that S1 neurons can be part of cross-modal memory networks.

758.13

INHIBITION OF LOCUS COERULEUS NEURONS BY SPONTANEOUS AND INDUCED FRONTAL CORTEX ACTIVITY D.A. Wilson*[1], A. Hervé-Minvielle, D. Robinson and S.J. Sara, Institute des Neurosciences, Univ Paris VI, Paris, 75005, France and [1]Dept. Zoology, Univ. Oklahoma, Norman, OK, USA

Given the neuromodulatory action of norepinephrine throughout the forebrain, identification of central structures influencing locus coeruleus (LC) activity can have important implications for our understanding of the role of this system in many aspects of information processing Recent evidence has shown that a small population of neurons in the medial precentral region of frontal cortex (FC) of the rat can be antidromically driven by LC stimulation; suppression of activity in these cells results in an enhancement of tonic LC activity (Sara & Hervé-Minvielle, *PNAS*, in press). The present study further examined the influence of FC activity on LC neurons in ketamine-anesthetized rats. Phasic LC unit responses to FC activity were examined using two methods. 1) Spontaneously active, multiple single-units were recorded simultaneously from FC and LC. Cross-correlation analysis examining LC unit activity as a function of FC unit activity, frequently showed a transient suppression of LC firing 25-50 ms after the FC unit discharge. 2) Patterned electrical stimulation of the FC produced a transient supression of LC activity, often followed by enhanced discharge at stimulus offset. Stimulation of the FC outside of the medial precentral region produced no, or much less, suppression. Together these data show, in addition to the previously demonstrated tonic influence, a phasic influence of a circumscribed region of FC on LC activity.

758.14

The effect of Δ^9-THC on neuronal activity in the frontal cortical-basal ganglia system during a delayed match to sample task in rats. *J-Y. Chang*, M.G. Laubach, A.B. Kirillov and D.J. Woodward, Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University. Winston-Salem, NC 27157.*

Marihuana is currently one of the most prevalent substances of abuse. Its aversive effects include a disruption of motoric and cognitive abilities. Cannabinoid receptors are densely concentrated in the frontal cortical and basal ganglia of rats, suggesting that behaviors that are dependent on the cortical-thalamic system should be sensitive to the effects of the cannabinoids. The present study employed ensemble recording to examine the effects of Δ^9-tetrahydrocannabinol (Δ^9-THC) on neuronal activities in medial frontal cortex (mPFC), striatum (STR) and substantia nigra pars reticulata (SNr) during performance of a delayed-match-to-sample-task. Rats were instructed to press a right or left sample lever and to then execute nose-poke responses a delay period (~15 seconds). The first nose poke after the delay period resulted in the presentation of both levers. Pressing the same lever as in the sample phase resulted in a reward, whereas pressing the lever opposite to that presented in the sample phase resulted in a time-out period. Single neuron and ensemble activities were analyzed by using peri-event histograms, joint peri-event time histograms and classification trees (CART). Alterations in spike activity associated with behavioral events in the sample, delay, and match phases were observed in mPFC, STR, and SNr. Δ^9-THC at doses of 0.5-1.0 mg/kg (i.p.) increased the occurrence of errors in the task in parallel with alterations in neuronal activity during the sample, delay and match phases. The data suggests that the frontal cortical-basal ganglionic system is part of neuronal circuitry through which the cannabinoids may influence attentional and memory process. *Supported by DA2338 W.W.W .server: http://biogfx.bgsm.wfu.edu.*

758.15

INVOLVEMENT OF GABAERGIC PROJECTIONS IN SYNCHRONOUS OSCILLATIONS IN CULTURED CORTICAL NEURON NETWORKS FIRING; BICUCULLINE EFFECTS FREQUENCY OF OSCILLATION. K.Kobayashi[1]*, K. Umezawa[1], A. Nozawa[1], K.Muramoto[1], M.Kawahara[1], E. Maeda[2], A.Kawana[2], Y. Kuroda[1]
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Synchronous oscillation of neuronal activities has been suggested to be important for sustaining firing to reinforce activity-dependent changes of synaptic plasticity in memory circuits (Kuroda et al Soc.Neurosci.Abstr. 20, 801, 1994). We have found that dissociated rat cortical neurons make networks with high synaptic convergence in culture and that the networks fire spontaneously in synchronous oscillation (Kuroda et al. Neurosci.Let.,135, 255,1992,). The existence of GABAergic neurons and synapses in the culture was investigated by immunocytochemistry. After fixation, approximate 10-15% of neurons were stained with both anti-GABA antibody and with Anti-GAD antibody. The proportion of GABAergic neurons in the primary culture is thus very similar to that of rat visual cortex in vivo. Anti-GAD also stained many small dots around neurons and their dendrites. When double-staining with anti-synaptotagmin antibody was carried out, many small dots of synaptotagmin staining were also stained by GAD antibody, indicating existence of abundant GABAergic terminals. Since inhibitory postsynaptic currents are also found in some neurons in the network (Robinson et al. J. Neurophysiol. 70, 1606,1993), the effects of a GABA antagonist on the synchronous oscillation of burstings were observed using fura-2 multi-site Ca fluorometry. Application of bicuculline decreased the frequency of synchronous oscillations suggesting a contribution of GABAergic input to the network activity.

758.16

THE GABAB RECEPTOR, INPUT SELECTIVE PRESYNAPTIC INHIBITION AND THE REPRESENTATION OF FAMILIARITY A.C. Tang* and M.E. Hasselmo Department of Psychology and Program in Neurosciences, Harvard University, Cambridge MA 02138.

Recent physiological studies in brain slice preparations of the piriform cortex (Tang and Hasselmo, *Brain Res.* 659: 75-81) demonstrate that the suppression of synaptic transmission (presynaptic inhibition) elicited by activation of GABAB receptors is selective for excitatory synapses between pyramidal cells, but not for afferent input to the cortex. We have used computational models of cortical function to study how this differential influence on afferent versus intrinsic inputs to principal neurons plays a role in the judgement of the novelty or familiarity of stimuli. The model contained coupled pairs of excitatory and inhibitory neurons, with full connectivity among the excitatory neurons. We focused on the transient responses of individual neurons, showing that the perceived familiarity of a pattern of afferent input can be represented by a quantity S -- the first derivative of the second inflection point of the neuronal response profile. Activity dependent presynaptic inhibition was crucial for an adequate representation of perceived familiarity in this model. Modeling allowed manipulation of network parameters corresponding to the presynaptic GABAB receptor density and the concentration of exogenous GABAB agonist (baclofen, used clinically to treat spasticity). Results show that either decreased GABAB receptor concentration on the presynaptic terminals, or increased exogenous GABAB agonist concentration, reduces perceived familiarity within the model. These results are consistent with the effect of chronic baclofen administration on rat behavior in an odor habituation task (Tang and Hasselmo, *Behav. Brain Res.* in press), the loss of recognition memory as a result of baclofen overdose, and the loss of GABAB receptors in Alzheimer's patients.
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758.17

Basolateral amygdaloid neuron responses in rats performing a spatial maze task. W.E. Pratt* & S. J. Y. Mizumori, Dept. Psychol., University of Utah, Salt Lake City, UT 84112

Much research involving the nucleus accumbens (NA) has suggested that it is a crucial site for the modulation of reward and spatial learning. Previously, we have reported the presence of cells with reward and spatial correlates within the NA (Lavoie & Mizumori, 1994). The pathway from the basolateral nuclei of the amygdala (BLA) to the NA may be important in the conduction of reward information. The present study investigated information encoding within the BLA which could then pass to NA neurons.

Single units in the BLA were recorded while animals performed a spatial memory task on an eight arm radial maze. Four arms consistently had low reward (1 drop chocolate milk) and four consistently had high reward (5 drops chocolate milk). Initial results (n= 43 cells, 1 rat, 2 passes in each of 2 hemispheres) indicate a variety of behavioral correlates. 14% of cells displayed location specificity. 30% showed an increase in firing rate of at least 20% (range:22-77%)when traveling outbound from the center of the maze relative to traveling inbound, and 14% increased inbound relative to the outbound rate (24-62% increase). 9% of cells decreased their rate 50% at the end of arms, with a corresponding increase once movement resumed. Three cells showed possible reward correlates; one fired only at the end of arms during trials, and the other two either showed a 50% increase or decrease which did not appear movement related.

These data, while not proving or disproving a role for the BLA in reward-related processes, suggest that the amygdala may encode more spatially-related forms of information than was previously imagined. These data illustrate for the first time the heterogenous nature of information coded in the BLA during performance of this spatial task. Consistent with lesion studies on conditioned place preference, the BLA may code reward associations within the spatial context in which learning occurs. [Supported by AG09299 and BNS 9120784]

758.18

SPATIAL REPRESENTATIONS OF DORSAL CAUDATE NEURONS OF FREELY-BEHAVING RATS. S. J. Y. Mizumori* & B. G. Cooper, Dept. Psychol., University of Utah, Salt Lake City, UT 84112

Experience-dependent visuospatial navigation requires not only continual update and modification of representations of our sensory surround as animals move through their environment, but also continual refinement of movement patterns. With respect to the latter process, ventral striatal neurons show dynamic spatial and reward properties that may be used to direct navigational behavior (Lavoie & Mizumori, 1994). This study extends our striatal analysis by assessing the contribution of the caudate nucleus to control of navigational behaviors.

Rats (9-12 mo old) performed multiple spatial memory trials on a radial maze. 192 neurons were recorded in dorso-rostral caudate nucleus of 7 rats. Consistent with Wiener (1993), a variety of spatial correlates were observed. Some spatial cells (n=8) showed clear directionally-specific, spatially-localized firing. Another type of spatial coding was shown by 1 cell which exhibited mnemonic, directional heading properties: it fired when the animal's head was oriented in a preferred direction in space, regardless of the animal's location. Such directional firing could be maintained in the absence of visual cues or voluntary movement. 10 other cells also showed directionally-specific firing, but the firing occurred relative to the maze configuration. Additional spatial cells selectively fired when animals made right and/or left turns (n=12); 3 of these displayed turn correlates only when animals occupied certain locations. Thus, caudate spatial messages are coded within multiple reference frames. Also, these data are consistent with primate studies which show that caudate neurons code contextually-dependent, nonmotor information relevant to spatially-guided learned behaviors. [Supported by AG09299 and BNS 9120784]

758.19

Intra-amygdala infusion of APV blocks auditory evoked potentials in the lateral amygdala and thalamo-amygdala transmission, but spares cortico-amygdala transmission. M. T. Rogan* and J.E. LeDoux, Center for Neural Science, New York University, NY, NY 10003.

Thalamic and cortical amygdala-fugal pathways have been implicated in fear conditioning with an acoustic conditioned stimulus. Since infusion of NMDA-antagonist APV in the amygdala disrupts fear conditioning, we studied the effect of APV infusion on neural transmission in the thalamo-amygdala and cortico-amygdala pathways, and on auditory evoked potentials in the lateral amygdala (LA). Urethane anesthetized rats were fitted with an auditory speaker for the delivery of a calibrated acoustic stimulus, and two stimulating electrodes: one in the medial division of the medial geniculate body and posterior intralaminal nucleus (MGm/PIN), and one in the TE3 region of auditory cortex. Averaged evoked potentials (EPs) elicited by these 3 stimuli (auditory-EPs, MGm/PIN-EPs, and TE3-EPs) were measured in the lateral amygdala with a steel recording electrode equipped with an attached micropipet (50μ OD) loaded with APV (50μM). After stable baseline measurements were obtained, .5μl of APV was infused, and all EP measurements were monitored at 15 min intervals. APV infusion reduced the slope of the auditory-EP and MGm/PIN-EP at least 75% from baseline levels, and these responses showed recovery with time. APV infusion had no effect on TE3-EP, which remained stable throughout the measurement period. These results indicate that auditory-evoked activity in LA and transmission through thalamo-lateral amygdala synapses are both largely dependent upon NMDA-receptor function. In contrast, transmission through cortico-amygdala synapses is not dependent on NMDA-receptor function. Supported by MH38774, MH46516, MH00956, MH10919.

758.20

THE AMYGDALA IS NECESSARY FOR THE INITIAL ACQUISITION BUT NOT FOR MAINTAINENCE OF DISCRIMINATIVE AVOIDANCE BEHAVIOR IN RABBITS. **A. Poremba* and M. Gabriel. Dept. of Psych. and Beckman Institute, Univ. of Illinois, Urbana, IL 61801.**

Bilateral electrolytic amygdala lesions block acquisition of discriminative avoidance conditioning wherein rabbits learn to step in a large activity wheel upon hearing a footshock-predictive tone conditional stimulus (CS+, 1 or 8 kHz) and to ignore a different tone (CS-, 1 or 8 kHz), not predictive of shock (Poremba and Gabriel, Soc. Neurosci. Abstr., 17:325, 1991). Here we asked whether the circuit necessary for the initial acquisition of the avoidance behavior is also involved in maintaining performance of the well-learned behavior. Rabbits (n=6) given bilateral intra-amygdaloid microinjections (.5 μl) of the $GABA_A$ agonist, muscimol (1 μmol), showed no signs of learning, either in terms of discrimination between CS+ and CS- or in terms of response incidence relative to a preliminary training session in which the CSs and the footshock were explicitly unpaired (p<.02). Rabbits given saline (vehicle) microinjections did develop discrimination (p<.02). Next, all rabbits (n=11) were trained to asymptotic performance and received 3 additional training sessions (overtraining). After this training, muscimol microinjections then significantly reduced performance relative to saline microinjections (p<.05). Finally, the rabbits received an additional seven days of training (n=4) or seven days of rest (n=5) followed by a muscimol and a saline session. In contrast to the findings after three days of overtraining, no significant reduction of avoidance responding was found. These results suggest that the amygdala is necessary for the initial acquisition of the discriminative avoidance behavior. Merely the passage of time after overtraining renders the amygdala unnecessary for maintenance of the avoidance behavior. (Support: NINDS grant NS26736 to MG).

758.21

PREDICTION OF LEARNED BEHAVIOR FROM ELECTRO-PHYSIOLOGY: DIMINISHED CONTRIBUTION OF LIMBIC THALAMUS TO AVOIDANCE RESPONDING AFTER EXTENSIVE OVERTRAINING IN RABBITS. **M. Gabriel*, M. Hart, A. Poremba. Dept. of Psych. and Beckman Institute, Univ. of Illinois., Urbana, IL 61801.**

Neurons in the limbic (anterior and medial dorsal) thalamic nuclei exhibit training-induced multi-unit activity (TIA), as rabbits learn to avoid a foot-shock by stepping in an activity wheel upon hearing a shock-predictive tone (CS+, 1 or 8 kHz), and to ignore a different tone (CS-, 1 or 8 kHz) not predictive of shock. The TIA, increased tone-elicited discharges (excitation), which are greater in response to the CS+ than to the CS- (discrimination), reaches maximal levels in the limbic nuclei as behavioral learning reaches asymptote (see Gabriel, M., in Neurobiology of Cingulate Cortex and Limbic Thalamus, 1993, Birkhauser: Toronto, 478-523). Bilateral electrolytic limbic thalamic lesions block learning (op. cit, 1993). The fact that TIA amplitude declines gradually during post-criterial training (overtraining) suggested that the contribution of limbic thalamus to the learned behavior may diminish during overtraining. Here rabbits (n=5) given training to behavioral asymptote followed by limbic thalamic lesions showed significantly impaired post-lesion retention, relative to sham lesion controls (n=4), as measured by avoidance response performance during extinction testing (p< .04) and re-acquisition to criterion (p< .04). Ample retention was exhibited by other rabbits (n=5) given lesions after receiving training to criterion plus ten days of overtraining. This performance did not differ from that of similarly trained sham lesion controls (n=5). Thus, the limbic thalamic nuclei are involved in original learning and maintained performance of discriminative avoidance behavior, but are not critical for the behavior in highly overtrained rabbits. (Supported by NINDS grant NS26736 to MG).

LEARNING AND MEMORY: PHYSIOLOGY IV

759.1

MUTUAL INHIBITION OF NEURAL PATHWAY SYSTEMS DURING FORWARD AND BACKWARD CONDITIONING SUGGESTS SYMMETRICAL ATTENUATING MECHANISM DURING ASSOCIATIVE LEARNING. T.Korhonen*, T.Ruusuvirta and J.Arikoski, Dept.of Psychol.,Univ.of Jyvaskyla, P.O. Box 35, 40351 Jyvaskyla, Finland.

Forward and backward conditioning procedures were used for an evaluation of the order effect of the conditioned (CS) and unconditioned stimulus (UCS) presentation. Six cats were first classically conditioned using tone-CS (1500 ms) delay paradigm in which a rewarding electrical stimulation train (500 ms) of the lateral hypothalamus served as the UCS. Both behavioral (head movements) and evoked neural responses were recorded in hippocampal areas (CA1, dendate fascia and subiculum) in freely moving cats. The result showed that during the forward pairing both the head movements and unconditioned evoked responses were significantly attenuated compared to to the UCS-alone presentations. Correspondingly, the responses to the CS (short latency, alpha responses) were significantly attenuated during the backward sessions compared to the CS-alone presentations. These findings suggest that the preceding stimulus leaves a temporal trace the effect of which temporally overlaps the subsequent stimulus. An assumption of the local postsynaptic interaction might explain the mutual inhibition effect of the converging CS and UCS pathways found in the present study. This conclusion was also supported by an observation of the specific modifying effect the UCS pathway on the CS pathway and vice versa.

759.2

PRESYNAPTIC INHIBITION: A TEST OF ITS POTENTIAL ROLE IN BLOCKING CLASSICALLY CONDITIONED REFLEX POTENTIATION AT CERTAIN INTERSTIMULUS INTERVALS. R.G. Durkovic*. Dept.Physiol., SUNY Health Sci. Ctr., Syracuse, NY 13210.

In spinal cat classically conditioned flexion reflex potentiation over spinal circuits activated by large myelinated (A-beta) cutaneous fibers occurs when the unconditioned stimulus (US) onset slightly precedes conditioned stimulus (CS) onset, but not when the CS and US completely overlap in time. Presynaptic inhibition of A-beta afferents of the US by CS inputs has been hypothesized as a mechanism responsible for blocking A-beta conditioned potentiation in paradigms with complete overlap of CS and US (Onifer and Durkovic, J. Neurosci. 8:502). The present study tested for evidence of presynaptic inhibition of the US (superficial peroneal (SP) nerve terminals) by saphenous (S) nerve inputs (the CS) by examining changes in SP primary afferent terminal thresholds following S stimuli (i.e., tests of primary afferent depolarization, PAD, which parallels presynaptic inhibition: J.Physiol. 161:258).

In unanesthetized decerebrate T-10 spinal cats stimulation of the L-6 dorsal root entry zone with single pulses elicited antidromic potentials recorded from the SP nerve. Single S nerve pulses preceding spinal cord stimuli potentiated initial and depressed secondary (dorsal root reflex) potentials recorded from the SP nerve for at least 70 ms with maximum effects at 25-50 ms after the S pulse. Similar results followed each one of the 10 Hz pulses of the S nerve train employed as the CS.

These results supply evidence for presynaptic inhibition of US primary afferent terminals by CS inputs. This phenomenon may prevent the necessary A-beta associative inputs from reaching first order interneurons when CS and US completely overlap, thus preventing A-beta associative reflex potentiation. Supported by NSF grant IBN 9220206.

759.3

BEHAVIOURAL, NEUROCHEMICAL AND MORPHOLOGICAL CHANGES IN AGED RATS: COMPARISON WITH BASAL FOREBRAIN AND ENTORHINAL CORTEX LESIONS. G.A. Higgins*, J. Clapham, J. Murray, D.L Kirkby, H.T.R. Rupniak, H. McCleave, J.C. Barnes Pharmacology 2, Glaxo Research & Development Ltd, Ware, Herts, UK.

In Alzheimer's disease (AD) some of the earliest neurodegenerative changes include synapse loss in the molecular layer of the dentate gyrus, as measured by synaptophysin immunoreactivity (SI; Masliah et al (1994) Neurosci. Lett. 174: 67) and reductions in forebrain choline acetyltransferase (ChAT; Perry (1986) Brit. Med. Bull. 42: 63). We and others have found similar changes in rats following electrolytic lesions of the entorhinal cortex (EC) or AMPA neurotoxin lesions of the basal forebrain (BF). In the present series of studies we have characterised 3 groups of male, Lister hooded rats (young (Y) 3 months; middle aged (MA) 15 months; aged (A) 22 months) for behavioural, neurochemical or morphological differences to compare with those produced by BF or EC lesions.

Water maze testing revealed clear age-associated deficits in acquisition to find a submerged platform, which could be dissociated from changes in motivation /sensorimotor function. A subpopulation of A rats showed particularly poor acquisition e.g day 4: path lengths Y=282±44cm, A unimpaired (AU)=331±43cm, A impaired (AI)=653±14cm; $p<0.01$ vs. Y and AU groups. Unlike EC lesioned rats, A rats (including AI) showed no significant change in SI in the molecular region of dentate gyrus compared to Y rats. Furthermore reductions in ChAT activity were much smaller then those produced by BF lesions, and were more prominent in the striatum, rather than hippocampus/cortex. Thus in the present studies we have failed to find a simple correlation between age associated changes in cognitive performance with SI/ChAT, perhaps indicating that multiple factors are likely to contribute.

759.4

TOPOGRAPHIC DISTRIBUTIONS OF THE MEDIODORSAL THALAMIC NEURONS DURING ASSOCIATIVE LEARNING IN RATS. T.Oyoshi, H.Nishijo, T.Asakura, T.Kobayashi* and T.Ono. Dept. Physiol., Fac. Med., Toyama Med. & Pharmaceu. Univ., Toyama 930-01, and Dept. Neurosurg., Fac. Med., Kagoshima Univ., Kagoshima 890, Japan.

Neuronal activities were recorded from the mediodorsal thalamic nucleus (MD) of behaving rats during discrimination and learning of conditioned stimuli associated with or without reinforcements: sucrose solution, intracranial self-stimulation, and aversion (electric shock). The rats were trained to lick a protruding spout just after a conditioned stimulus to obtain reward or to avoid aversion. Of 122 MD neurons responding during the task, the activity of 13 correlated to licking (behavior-related), and that of 109 did not (behavior-nonrelated). Activity of behavior-related neurons increased just before licking only during the task, but not before spontaneous licking during the intertrial interval. These behavior-related neurons were mainly located in the lateral segment of MD, which has intimate anatomical connections with motor-related areas such as anterior cingulate and striatum. Of 109 behavior-nonrelated neurons, 97 responded differentially to conditioned sensory stimuli and the remaining 12 responded nondifferentially. These differential behavior-nonrelated neurons had the following characteristics: First, most neurons (90/97) responded to both auditory and visually conditioned stimuli. Second, most neurons (37/39) changed their responses plastically along with learning during the extinction and relearning trials. Third, most neurons (8/14) responded during the delay period, as well as during the conditioned stimulation period in the reward task with delay, in which delay was imposed between the conditioned auditory stimuli and the protrusion of a spout. These differential behavior-nonrelated neurons that had short latencies less than 300 ms were mainly located in the rostro-medial portion of the MD, which receives afferents from the basolateral nucleus of the amygdala, where various sensory information converges. The present results, along with previous anatomical studies, suggest the evidence of two limbic circuits; anterior cingulate-striatum-lateral MD (motor), and amygdala-medial MD-orbital cortex (emotional/motivational) circuits.

759.5

THE INVOLVEMENT OF NUCLEUS ACCUMBENS IN LATENT INHIBITION. I. Weiner√#, G. Gilad√, J. Feldon√#, √Dept. Psychology, Tel-Aviv University, Tel-Aviv, **Israel**. #Lab. Behav. Biol. Funct. Toxicol., Instit. Toxicol., Schorenstrasse 16, Schwerzenbach 8603, **Switzerland.** (ENA)

When an organism receives repeated presentation of a stimulus without any other consequences, it subsequently disregards this stimulus when it is followed by significant consequences, e.g., reinforcement. This is reflected in slower conditioning to this stimulus as compared to a novel stimulus and constitutes the phenomenon of latent inhibition (LI). LI is absent in rats and humans receiving the psychotomimetic, amphetamine, as well as in acute schizophrenic patients. Neuroleptic treatment restores LI in amphetamine-treated rats and schizophrenics. These results have led to the proposition that LI disruption models a cardinal cognitive deficit in schizophrenia, namely, an inability to ignore irrelevant stimuli. This proposition is strengthened by the fact that the neural substrates of LI parallel those implicated in the pathophysiology of schizophrenia, namely, the retrohippocampal region and the ventral striatum. The present experiments investigated the involvement of the two subterritories of the nucleus accumbens (NAC), shell and core, in LI. Electrolytic lesions of shell and core produced distinct effects on LI: shell lesion led to total disappearance of LI whereas core lesion only attenuated conditioning to a novel stimulus. Only shell lesion increased amphetamine-induced activity, suggesting that it produced increased dopamine (DA) transmission. This was further substantiated by the fact that the administration of a DA blocker, haloperidol, restored LI in shell lesioned animals. Thus, the capacity to ignore irrelevant stimuli critically depends on the integrity of the shell subterritory of the NAC (and therefore probably on the inputs received by this region from retrohippocampal structures), and an impairment of such capacity is subserved by increased mesolimbic DA transmission. In addition, these results provide the first behavioural demonstration for the functional specialization of the shell and core subterritories of the NAC.

759.6

DIFFERENTIAL LEARNING-RELATED ACTIVITY OF HIPPOCAMPAL SINGLE UNITS DURING CLASSICAL CONDITIONING IN THE RABBIT. R.L. Borgnis*, M.A. Seager and S.D. Berry. Center for Neuroscience Research and Department of Psychology, Miami University, Oxford OH 45056.

While it is generally accepted that hippocampal cells show plasticity during behavioral classical conditioning, unit/behavior analyses from several laboratories have indicated a surprising heterogeneity of responses among similar cell types both within and across hippocampal subfields (e.g., Berger *et al.* 1983; Ranck 1973; Weiss *et al.* 1993). However, much of the research has been done in a single aversive paradigm (rabbit NM conditioning). To expand these findings, we recently showed that multiple unit activity recorded from CA1 corresponded to the behavioral response in the appetitive conditioned jaw movement paradigm (CJM; Oliver, Swain & Berry 1993). Given the variety of potential single unit response profiles (as in NM conditioning), we decided to undertake single unit analyses of various hippocampal cell types during CJM.

New Zealand White rabbits were anesthetized (ketamine 50mg/kg and xylazine 10mg/kg) and unilaterally implanted with a stainless steel microelectrode. A capped metal cylinder was cemented into place over the contralateral hemisphere that would later hold a microdrive unit containing both single and multiple unit electrodes. Rabbits were trained for two days (54 trails per day) in CJM prior to attachment of the microdrive on the third day. Hippocampal unit responses and field potentials were recorded from both hemispheres. Some rabbits were trained in NM/CJM concurrent discrimination.

Our results show that cells within the same electrophysiological classification (e.g., spike widths >0.5 ms) respond differently during CJM, some showing diffuse increases in firing rate to the tone, others showing periodicities similar to the rhythmic jaw movement response. Some units are from animals that were trained in a concurrent NM and CJM discrimination task to assess whether the same cell responds differently during performance of the two distinct behavioral responses.

759.7

Hippocampal CA1 Single Neuron Activity During Trace Eyeblink Conditioning C. Weiss,* M.A. Kronforst, and J.F. Disterhoft, Dept. of Cell and Molecular Biology, Northwestern University Medical School, Chicago, Il. 60611

Multichannel recordings were used to monitor the activity of several hippocampal CA1 pyramidal cells simultaneously during trace eyeblink conditioning in the rabbit (100 ms tone, 500ms trace, 150 ms airpuff). Control rabbits were presented with unpaired tones and airpuffs. The Datawave software package was used to separate the activity of single neurons from each electrode. Pyramidal cells were separated from interneurons according to spike width, spike rate and the presence of complex spikes. Activity during the tone, trace, UR (500ms after airpuff onset), and post trial (PT) periods (2s after the UR period) was compared with activity from an equal time window prior to tone onset using either one sample t-tests or the binomial test (for slowly firing cells). Changes were considered significant if $p \leq 0.01$.

Recordings were made from 113 CA1 pyramidal neurons from 11 rabbits during trace conditioning and from 23 neurons from 5 rabbits during unpaired stimulation. Many of the neurons (40%) had baseline firing rates ≤ 0.5Hz. During sessions with >60% CRs, 46% of the neurons exhibited a statistically significant change in firing rate during the trial period. Another 18% changed only during the PT period. In comparison to control rabbits, the conditioned rabbits were found to have a greater percentage of significantly affected neurons during the CS, trace and PT periods. The percentage of cells from conditioned and control rabbits that changed during the UR period was similar. Both excitation and inhibition occurred during the trial period, but inhibition predominated during the PT period.

The low number of significantly related cells, the great diversity of responses, and the large number of neurons with significant inhibition during the trial period suggest that information processing by the hippocampus is more complex during hippocampally-dependent conditioning than during hippocampally-independent conditioning (cf. Berger et al., 1983).

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759.8

INCREASED SYNAPTIC RESPONSIVENESS BETWEEN CA3 AND CA1 AFTER TRACE EYEBLINK CONDITIONING RECORDED *IN VITRO*. J.M. Power*, L.T. Thompson, J.R. Moyer, Jr., and J.F. Disterhoft, CM Biology, Northwestern University Medical School, Chicago, IL 60611.

Thirty-four young (2 mo) female NZW rabbits received either trace conditioning (80 trials daily: 100 ms tone CS; 500 ms trace ISI; 150 ms corneal airpuff), pseudoconditioning, or were naive. Conditioned animals were trained daily until reaching a criterion of 80% CRs in a given session. Hippocampal slices were prepared 1 h or 24 h following the final training session. Field potentials were evoked by stimulation of the Schaffer collaterals, using a stainless steel bipolar electrode. Somatic and dendritic field potentials were recorded in CA1, 750 µm from the stimulating electrode. An input-output function for each slice was generated using a 100 µA current and a range of stimulus durations (20 - 600 µs) encompassing subthreshold to maximal population spike responses.

The maximal population spike amplitude was 39% greater in slices from conditioned animals sacrificed 1 h after acquisition and 24% greater in slices from conditioned animals prepared 24 h after acquisition relative to naive controls. Additionally, the EPSP slope was found to be greater in slices prepared from conditioned animals 1 h post acquisition. Field potentials from naive and pseudoconditioned animals were indistinguishable. All recording and data reduction was performed **blind** to the conditioning task. Further analysis and experimentation are being done to decipher whether the enhanced population spike amplitudes result from altered synaptic efficacy, increased postsynaptic excitability, or both.

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759.9

CLASSICAL CONDITIONING USING NUCLEUS BASALIS STIMULATION PRODUCES ASSOCIATIVE CS-SPECIFIC DESYNCHRONIZATION OF AUDITORY CORTEX EEG. JS Bakin*, TS Bjordahl, and NM Weinberger, Center for the Neurobiology of Learning and Memory, and Department of Psychobiology, University of California, Irvine, CA 92717

Cholinergic system activity plays a critical role in learning and memory, cortical processing, and cortical plasticity (cf Weinberger 1993 *Current Opinion Neurobio*). Classical conditioning using an auditory conditioned stimulus (CS) produces specific long lasting plasticity in primary auditory cortex (ACx) receptive fields (RFs), resulting in retuning RFs to or towards the CS frequency (Bakin and Weinberger, 1990 *Br Res*). We hypothesized that this plasticity requires the convergent activation of acoustic thalamocortical and cholinergic modulatory inputs to the ACx (Weinberger et al, 1990 *CINS*). As an initial test of this model, we classically conditioned urethane anesthetized adult male rats, substituting electrical stimulation of the NB for footshock, as the unconditioned stimulus. Neuronal RFs and cortical EEG were simultaneously obtained from ACx ipsilateral to the NB stimulating electrode, and contralateral to the CS speaker. Here we report the effect of classical conditioning with NB stimulation on ACx EEG.

NB stimulation alone (500ms, 200Hz, 100-300μA) produced EEG desynchronization (14/14 subjects, $p<.0003$) that could be blocked by atropine (5/5, $p<.05$). Auditory tones alone did not (5/5, $p>.43$). During RF characterization following tone/NB conditioning (40 trials), the CS frequency ($p<.03$), but not non-CS frequencies ($p>.61$) produced EEG desynchronization. Sensitization controls did not develop CS-induced EEG desynchronization ($p>.26$), indicating that this effect is associative. This CS-specific EEG desynchronization could be detected 30 minutes post training, the longest interval tested.

These results demonstrate that convergent activation of acoustic and cholinergic inputs to ACx can support cortical plasticity, and might provide the basis for ACx RF plasticity during learning. Supported by NIDCD #DC02346 (NMW).

759.10

MULTIPLE-UNIT RECORDING OF THE INTERPOSITUS NUCLEUS DURING CLASSICAL CONDITIONING OF THE RABBIT EYEBLINK RESPONSE TO INTERMODAL (LIGHT AND TONE) STIMULI. J. A. Huska* and J. E. Steinmetz. Program in Neural Science and Department of Psychology, Indiana University, Bloomington, IN, 47405.

The interpositus nucleus (INP) is believed to be essential for the acquisition of a classically conditioned eyeblink response (CR) following pairings of either light or tone conditioned stimuli (CS), and an airpuff (US) to the cornea. Previous single-unit and multiple-unit recordings from the INP have revealed neuronal activity that precedes and models the CR. Allen and Steinmetz, 1994 found sites in cerebellar cortex that modeled CRs to two different tone frequencies, one of the tones signalling a long interstimulus interval (ISI), the other signalling a short ISI. The present study sought to determine whether recording sites in the INP could be found that would model CRs to stimuli from two distinct sensory modalities.

Rabbits were surgically implanted with multiple-unit electrodes in both the left and right INP and trained on each side with both tone and light CSs until reaching a criterion of 75% or more CRs for two consecutive days. In each session the rabbit received 120 CS-US pairings of a 350 ms CS that co-terminated with a 100 ms airpuff in a standard delay conditioning paradigm. Activity was recorded from the INP throughout training.

Preliminary findings indicate tone-related CR activity at sites within the INP, but no recording sites have modeled CRs to both tone and light CSs. Examination of the histological data reveals that thus far the sites sampled have been from lateral sections of the INP. These results would suggest that separate pathways exist through the INP for encoding the light versus tone CS-US association. We are currently recording from other deep nuclear areas in an attempt to localize sites where light CS-related activity can be found. Supported by NIMH grant # MH51178.

759.11

IMMUNOHISTOCHEMICAL EXPRESSION OF THE c-FOS PROTEIN IN THE SPINAL TRIGEMINAL NUCLEUS FOLLOWING PRESENTATION OF A CORNEAL AIRPUFF UNCONDITIONED STIMULUS. M.D. McEchron*, P. M. McCabe, T. J. Webber, E. J. Green, Janice M. Hitchcock, and N. Schneiderman, Behavioral Neuroscience Program, Dept. of Psychology, University of Miami, Coral Gables, FL 33124.

Previous work has shown that pairing a tone-conditioned stimulus (CS) with a corneal airpuff-unconditioned stimulus (US) produces reliable heart rate (HR) conditioning. A major goal in the neurobiological study of this HR conditioning paradigm is to localize potential sites of plasticity in the central nervous system (CNS) where CS and US information converge. The circuitry involved in the processing of the tone-CS has been examined in some detail, however, less is known about how airpuff-US information reaches critical sites of plasticity in the CNS. Therefore, the present study examined the expression of the c-Fos protein in the rabbit's CNS to determine which areas are activated by the presentation of the corneal airpuff US. In this study restrained awake rabbits received 100 presentations of a corneal airpuff. Brains were then removed and processed immunohistochemically for the c-Fos protein. In animals that received the airpuff, the ventral portion of the ipsilateral spinal trigeminal subnucleus caudalis (SVc) and interpolaris (SVi), and the dorsal raphe nucleus exhibited a greater number of c-Fos labeled cells per coronal section compared to control animals. Another group of animals was given microinjections of WGA-HRP in the medial nucleus of the medial geniculate (mMG) to determine if this critical area of the HR conditioning circuitry receives projections from SVc and SVi. These injections produced retrograde labeling in the same areas of SVc and SVi activated by the airpuff. Thus, a corneal airpuff US activates neurons in SVc and SVi which could then activate neurons in mMG. This provides additional evidence that CS and US information converge in mMG, an area believed to be a site of plasticity in the HR conditioning pathway. Supported by NSF IBN9222194 and NIH HL07426.

759.12

NEURAL EXCITABILITY MEASURED INTRACELLULARLY IN VIVO IN VENTRAL COCHLEAR NUCLEUS (VCN) AFTER BEHAVIORAL SENSITIZATION AND CONDITIONING. C. Woody*, X.F. Wang and E. Gruen. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Cats were studied before and after pairing 70 db click as CS with glabella tap and hypothalamic electrical stimulation (HS) followed 2.4 s by 70 db hiss as a DS. This order of presentation produced blink conditioning to the CS. When HS preceded the CS, tap and DS, sensitized responses to click and hiss were observed. An earlier study (Woody et al. NeuroReport 1994) showed greater activity increases to the stimuli after sensitization than conditioning. Further studies have tested unit excitability by measuring threshold currents for intracellular depolarizing pulses to initiate spiking. Percentages of neurons responding with increased activity 4-8 ms after click and 20-32 ms after the more slowly rising onset of hiss were also compared.

nA	ADAPTATION	SENSITIZATION	CONDITIONING
to fire	0.97±.36	0.57±.33	0.79±.33
response	n / %	n / %	n / %
to click	8 / 7	20 / 24	20 / 12
to hiss	3 / 3	13 / 16	12 / 7

The results suggest that the facilitation of activity in VCN cells is mediated by increases in excitability that are expressed postsynaptically in the neurons and potentiate their ability to respond. (Supported by Deafness Research Foundation.)

759.13

DIFFERENT NEURONAL ACTIVITIES IN ASSOCIATIVE FOREBRAIN REGIONS OF IMPRINTED AND NAIVE CHICKS. M. Bredenkötter* and K. Braun. Institute for Neurobiology, Magdeburg, Germany.

The medial part of the rostral neostriatum/hyperstriatum ventrale (MNH) in chicks is involved in auditory filial imprinting. 2-DG studies show that in imprinted chicks the MNH is strongly activated upon playback of the acoustic imprinting tone. Thus, we compared the activity of MNH neurons in imprinted and naive chicks evoked by either the imprinting tone or an unknown discrimination tone using freely moving or urethane anesthetized chicks.

In imprinted chicks the FFT-power of slow field potentials is increased during playback of the imprinting tone compared to the discrimination tone, whereas no differences are detectable between both stimuli in naive chicks. Single unit activity in imprinted chicks is drastically enhanced during playback of the imprinting tone in comparison to spontaneous activity ($p\leq0.002$) and during playback of the discrimination tone ($p\leq0.07$). The spike rate during playback of the imprinting tone is significantly higher than the spike rate during playback of the discrimination tone ($p\leq0.004$). In naive chicks no significant differences in neuronal activity could be observed between both tones and spontaneous activity.

Our results lead to the assumption that the MNH is also involved in memory recall. The single unit recordings in anesthetized chicks are in good agreement with the results from freely moving animals: there is an increase of neuronal activity in imprinted chicks but not in naive animals during playback of the imprinting tone in relation to spontaneous activity. While the discrimination tone evokes an increase of spike rate in the MNH of imprinted chicks, activity does not change significantly in naive controls. These results suggest that early acoustic experience during filial imprinting changes the neuronal responsiveness towards meaningful acoustic stimuli in the MNH.

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759.14

EXCITATORY SYNAPTIC TRANSMISSION IN TELENCEPHALIC *IMHV* SHOWS A HEBBIAN POTENTIATION ONLY IN EARLY NEONATAL CHICKS: A SLICE PATCH EXPERIMENT.

T. Matsushima*[1,2] and K. Aoki[1],

1. Life Science Institute, Sophia Univ., Tokyo 102, and 2. School of Agricultural Sciences, Univ. of Nagoya, Nagoya 464-01, JAPAN.

Properties of local synaptic transmission were examined by whole-cell patch recording in slices of telencephalic hyperstriatum ventrale (the *IMHV* region) of neonatal quail chicks at 1-3 days (young) and 7-10 days (old) post-hatch. Postsynaptic current responses to local electrical stimuli were composed of an early excitatory and a late inhibitory components. The early EPSC proved to be mediated by monosynaptic activation of DNQX-sensitive glutamate receptors, while the late IPSC was mostly due to polysynaptic activation of bicuculline-sensitive GABA receptors. Upon depolarization of the postsynaptic neuron at -45 mV or above, a tetanic stimulation of low frequency (5 Hz x 100-300 pulses) induced a transient slow inward current. The slow inward current was strongly depressed at -70mV and AP5-sensitive, thus proved to be NMDA-ergic. The tetanic stimulation also induced a long-term potentiation of the DNQX-sensitive fast EPSC only in young chicks below 3 days. In older chicks, however, tetanus similarly induced the NMDA-ergic slow inward, though failed to initiate LTP. Such a temporal window of synaptic plasticity in the *IMHV* could represent **sensitive period** of early learning processes such as filial imprinting and passive avoidance learning. A change in mechanisms downstream to the Ca^{2+} influx via NMDA receptors could be responsible for terminating the sensitive period.

759.15

RESPONSE CHARACTERISTICS OF VENTRAL TEGMENTAL NEURONS IN THE AWAKE RABBIT. F.A.Guarraci* and B.S.Kapp, Dept. of Psychology, Univ. of Vermont, Burlington, VT 05405.

A great deal of research has indicated that the mesotelencephalic dopamine (DA) system plays an important role in reward and aversive processes. Nevertheless, there has been a paucity of research describing in detail the sensory response characteristics of single DA neurons within the ventral tegmental area (VTA) of awake animals, particularly in response to stimuli predictive of aversive events. The present experiment is one of a series designed to investigate the response characteristics of VTA neurons to a variety of novel auditory stimuli (e.g., pure tones, white noise) and to auditory stimuli which predict aversive events in the awake rabbit. Single DA neurons within the VTA were isolated and identified according to established criteria: spontaneous rate of 0.8-10Hz and >2.0msec spike duration. In the absence of stimulus presentations, the spontaneous activity of these neurons was characterized by single spikes interspersed with bursts of 2-6 spikes with a mean burst interspike frequency of approximately 50msec. In response to auditory stimuli, three distinct response patterns were observed among different VTA neurons. The first was an increase in activity within 100msec of stimulus onset which was often characterized by a spike burst. The second response pattern was characterized by a marked inhibition to stimulus onset which outlasted the duration of the stimulus. The third pattern was characterized by a lack of response to auditory stimuli. Additional, preliminary data suggest that these three response patterns also occur in response to Pavlovian aversively-conditioned auditory stimuli. These results suggest that DA neurons within the VTA of the rabbit are responsive to novel auditory stimuli as well as to auditory stimuli predictive of aversive events.

759.16

ANALYSIS OF GENE EXPRESSION ASSOCIATED WITH MEMORY CONSOLIDATION IN RATS USING THE DIFFERENTIAL DISPLAY METHOD. A-Min Huang*[1] and Eminy H. Y. Lee[2]. Graduate Institute of Life Sciences, National Defense Medical Center[1] and Institute of Biomedical Sciences, Academia Sinica[2], Taipei, Taiwan, R. O. C.

Involvement of gene expressions associated with memory formation has been examined in learning paradigms for some invertebrates and vertebrates. However, little is known in the paradigm of inhibitory avoidance learning in rats. In this study, a PCR-related procedure based on the method of mRNA differential display was used to analyze differential gene expressions related to memory consolidation. Total hippocampal RNAs isolated from animals showing poor and good retention scores were used for comparison. Three 3'-end primers ($T_{12}AG$, $T_{12}GC$, and $T_{11}CA$) and four 5'-end arbitrary 10-mers were used. Each primer pair generates an average of 50-70 cDNA bands and most of the bands between individuals are identical. Several bands were found to be differentially expressed between poor and good memory rats. In one particular case, only a 3'-end primer ($T_{12}AG$) was used for PCR amplification. Two cDNA bands, designated as upper and lower band, showed differential expression between individuals of poor and good memory. These two bands were separately recovered from the gels, cloned into a TA vector, and sequenced. Both of them showed 90% homology with the 3'-end cDNA sequence of the glial fibrillary acid protein (GFAP). For further examination, 13 poor and 13 good memory individuals were compared. In the poor memory group, the lower cDNA band was found in 12 individuals. However, only 5 individuals in the good memory group showed this band. These results suggest that two different GFAP-like transcripts exist in the population and one of them may be associated with memory consolidation. cDNA clones of these two different transcripts will be isolated from the individual hippocampal cDNA library and the differences will be characterized.

759.17

ENHANCED HIPPOCAMPAL CRF GENE EXPRESSION ASSOCIATED WITH MEMORY CONSOLIDATION IN RATS. E.H.Y. Lee*, A.M. Huang and K.S. Tsuei Inst. Biomed. Sci., Academia Sinica, Taipei 115, Taiwan, R.O.C.

Corticotropin-releasing factor (CRF) was found to produce various behavioral changes other than its neuro-endocrine function. We have previously found that intra-hippocampal injections of CRF significantly improve memory retention of an inhibitory avoidance learning task in rats and CRF antagonist prevents this effect. Other laboratories have also found that i.c.v. injections of CRF enhance acquisition/learning in different learning paradigms in rats and mice. CRF gene expression was demonstrated in various brain regions including the hippocampus. The present study used the quantitative RT-PCR method to examine CRF gene expression in the memory consolidation process. One-way inhibitory avoidance learning paradigm was adopted. Results indicated that during the early phase of memory consolidation, which is 1 hr and 3 hr after the training procedure, there was a marked increase of CRF mRNA level in the hippocampus in animals showing good memory when compared with those showing poor memory. These results suggest that CRF gene expression is enhanced during the memory consolidation process.

759.18

ALTERATION IN THE ACTIVITY OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II ASSOCIATED WITH SPATIAL LEARNING IN RATS. S.E. Tan* and K.C. Liang+. *Dept. Psychology, Kaohsiung Med. Coll., and +Dept. Psychology, Natl. Taiwan Univ., Taiwan, R.O.C.

Protein phosphorylation plays a major role in neuronal plasticity as well as in behavioral learning processes. CaM-kinase II (Calcium/calmodulin-dependent protein kinase II) is one of the major brain protein kinases involve in learning and memory processes. The present study examined the role of CaM-kinase II in spatial behavioral learning.

Sprague-Dawley rats were trained in the Morris Water Maze. Three experimental groups recieved 5-, 2-, or 1-day training; and a control group. The hippocampal CaM-kinase II activities were analysed. The results showed that the activation of hippocampal CaM-kinase II (measured in term of the percentage of Ca^{++}-independent activity) was proportional to the extensiveness of behavioral training. There was a significant difference between the control and 5-day training groups in the percentage of Ca^{++}-independent activity ($p<.05$). There were no differences in the Ca^{++}-independent activity between the control and 1-day training groups, as well as in the total CaM-kinase II activity across the groups. Intra-hippocampal injection of a specific CaM-kinase II inhibitor, KN-62 (1-[N,O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosine]-4-phenyl-piperazine), hindered the spatial learning significantly ($p<.01$). The Ca^{++}-independent activity of CaM-kinase II in the KN-62 treated group was significantly lower than that in the vehicle control ($p<.01$). Our results indicate that the activation of CaM-kinase II is correlated with spatial learning.

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759.19

BEHAVIORAL STRESS IMPAIRS HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) THROUGH ACTIVATION OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS. J. J. Kim*[1], M. R. Foy[2] and R. F. Thompson[1], [1]Neurosciences Program, Univ. of Southern Calif., Los Angeles, CA 90089-2520 and [2]Psychology Dept., Loyola Marymount Univ., Los Angeles, CA 90045-2669.

Behavioral stress is known to impair various learning and memory tasks, and also to impair hippocampal LTP. Our previous work suggested that stress may occlude subsequent LTP development by elevating the basal synaptic transmission level. Since the induction of LTP in the hippocampus (e.g., area CA1) is known to require activation of NMDA receptors, we tested whether NMDA antagonists can block the stress effect on subsequent LTP.

Adult Long-Evans male rats (290-350 g) were administered intraperitoneally (i.p.) with either CGP39551 (30 mg/kg), a competitive NMDA receptor antagonist which crosses blood brain barrier, or saline 2 hours prior to stress. The stress consisted of 60 tailshocks (1mA, 1-s, 30-90-s apart) while restrained. Following stress, hippocampal slices were prepared in a standard manner. When initial slopes of field-EPSPs were examined in stratum radiatum in CA1 following stimulation of Schaffer collateral/commissural fibers, we found that slices from saline-stressed animals exhibited significantly impaired LTP ($107.9\pm4.9\%$; mean $\pm$ sem) after tetanus in comparison to slices from saline-control ($141\pm6.4\%$), CGP39551-control ($140.4\pm 8.0\%$) and CGP39551-stressed ($151.1\pm9.8\%$) animals. The CGP39551 effect appears not to be due to anxiolytic properties since slices obtained from animals injected with the anxiolytic drug diazepam (5 mg/kg i.p.) and then stressed were still impaired in LTP. Thus, our results suggest that the process by which stress impairs LTP appears to be mediated through NMDA receptor activation. Supported by grants from NRSA 1F32MN10521-01 BNR to JJK, LMU to MRF, and NSF IBN9215069, NIA AF05142 and Sankyo to RFT.

759.20

PRENATALLY-INITIATED LEAD (Pb) EXPOSURE IMPAIRS HIPPOCAMPAL LTP IN THE AWAKE RAT. M.E. Gilbert*[1,2], C.M. Mack[2], and S.M. Lasley[1], U. North Carolina, Chapel Hill, NC 27599, US EPA, RTP, NC 27711, and U.Ill. Coll. Medicine, Peoria, IL 61656.

Childhood lead (Pb) exposure has been associated with impaired cognitive function. Previously, we employed the hippocampal long-term potentiation (LTP) memory model system and demonstrated an increase in the threshold for LTP induction in the urethanized Pb-exposed rat. The present study examined the induction and time course of decay of LTP in the dentate gyrus of the conscious rat chronically exposed to Pb. Prior to mating, female rats received 0.2% Pb-acetate in the drinking water and male offspring were maintained on this solution. As adults, animals were prepared with chronic stimulating/recording electrodes in the hippocampus. Following recovery from surgery and the establishment of stable field potentials evoked by single pulse stimulation (0.1 ms) of the perforant path, a pretrain input/output (I/O) function was collected. LTP was then induced by delivering 10 train pairs (4 pulses/pair at 400 Hz), 200 ms apart, with an intertrain interval of 10 s at 6 stimulus intensities (200-1000μA). Three additional I/O functions, 1 hr, 1 day, and 1 wk following tetanization were obtained to monitor induction and decay of LTP. Comparison of pre- and post-train I/O functions revealed a significant enhancement of PS amplitude in the control group, but no difference from pretrain levels in the Pb-exposed group at any timepoint. These data suggest that the detrimental effects of developmental Pb exposure on cognitive function may be due to its interference with the synaptic processes underlying LTP. (Supported by [1]NIH ES06253 and [2]US EPA).

759.21

Hippocampal LTP and Spatial Learning are Attenuated by $GABA_B$ Receptor Blockade in the Rat

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This series of experiments assessed the role of $GABA_B$ receptors in the induction of long-term potentiation (LTP), and spatial learning and memory using three different tasks. In urethane-anaesthetized rats, theta burst stimulus trains were applied to the angular bundle and recorded in the dentate gyrus. Control LTP was attenuated from a mean of 145% to 57% in CGP 46381 treated rats ($t_{(13)}$=2.502,p=0.028,n=14).

We assessed the performance of rats on several spatial learning and memory tasks in CGP 46381 and saline treated rats. We found that the working memory performance of highly trained rats on the eight arm radial maze was unaffected by CGP 46381. There was also no effect of $GABA_B$ receptor blockade on learning in the eight-arm maze using a repeated acquisition paradigm. However, when we tested spatial learning in naive rats using the Morris water maze, we found that CGP 46381 treated rats (n=12) took significantly longer ($p<0.025$) to learn the location of the escape platform than controls(n=11). In addition, CGP 46381 treated rats travelled a greater distance over the acquisition and probe trials than did control rats. These data support our hypothesis that $GABA_B$ receptor blockade suppresses hippocampal LTP *in vivo* and impairs spatial learning in a task where stress may be important.

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LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS XI

760.1

THE CONTRIBUTION OF THE MEDIAL SEPTUM TO THE FUNCTIONS OF THE HIPPOCAMPAL SYSTEM IN RATS. R. NUMAN.* Psychology Department, Santa Clara University, Santa Clara, CA 95053.

In people, consensus now favors the view that the septohippocampal system regulates declarative memories. However, as non-human animals do not have language ability, it has been difficult to identify the analogue of declarative memory in these species. We review experiments from our laboratory on the effects of medial septal lesions on the behavior of male Long-Evans hooded rats, and conclude that rats may 'declare' what they know in terms of their volitional behavior, and that the working memory for such voluntary goal directed behavior is disrupted by medial septal lesions.

In one experiment (Numan and Quaranta, 1990), medial septal lesions impaired a left-right operant delayed alternation task which appeared to be caused by a deficit in working memory. In a second experiment (Numan and Klis, 1992) we found that medial septal lesions facilitated performance on a delayed but cued go/no-go stimulus working memory task, suggesting that the lesions do not produce a general working memory impairment. In a final experiment (Numan, 1994) we found that medial septal lesions impaired performance on a delayed go/no-go response alternation task suggesting that the deficit is specific to response working memory, and that spatial task requirements are not necessary for the expression of such a deficit.

We suggest that the septohippocampal system functions as a response intention--response outcome working memory comparator and that while damage to the system impairs the working memory for voluntary behavior, it may also lead to a compensatory reliance on, and hence a potential enhancement of the functions of, exteroceptive cue based memory systems.

760.2

LOSS OF NUCLEUS BASALIS MAGNOCELLULARIS, BUT NOT SEPTAL, CHOLINERGIC NEURONS CORRELATES WITH PASSIVE AVOIDANCE IMPAIRMENT IN RATS TREATED WITH 192-SAPORIN. R.G. Wiley*, Z.-J. Zhang, T.G. Berbos and C. Wrenn, Lab of Experimental Neurology, VAMC, and Vanderbilt University, Nashville, TN 37212-2637.

Intraventricular injection of the immunotoxin, 192-sap, that selectively destroys neurons expressing the low affinity neurotrophin receptor, $p75^{NGFr}$, efficiently and selectively destroys the cholinergic neurons of the basal forebrain (CBF). In the present study, we sought to determine if there was a correlation between the degree of CBF neuron loss and alteration in passive avoidance behavior. Anesthetized, adult, male Sprague-Dawley rats were stereotactically injected with 4 μg of either 192-sap or OX7-sap, a control immunotoxin that recognizes the Thy 1 surface antigen and destroys cerebellar Purkinje neurons. 6-8 weeks later, immunotoxin and naive control rats were tested on a step-through passive avoidance paradigm. After behavior testing, all rats were sacrificed and brain sections processed for histochemical demonstration of AChE and immunohistochemical demonstration of $p75^{NGFr}$. The numbers of neurons in specific regions of the CBF were counted from the $p75^{NGFr}$ staining and the intensity of dorsolateral neocortical staining for AChE were assessed using image analysis. The magnitude of cell loss was similar (67-70%) for the entire CBF , the Nbm and septum/DBB. The severity of passive avoidance impairment was significantly correlated to cell loss in the entire CBF (r=0.748, 23 df, $p<0.001$) and in the Nbm (r=0.778, 23 df, $p<0.001$) but not the septum/DBB (r=0.419, 23 df, $p>0.05$). Behavioral impairment also correlated significantly to loss of cortical AChE staining intensity ipsilateral to the intraventricular injections (r=0.796, 15 df, $p.<0.001$). These findings show that loss of Nbm, but not of septum/DBB, cholinergic neurons is proportional to impairment in passive avoidance behavior suggesting a role for Nbm-neocortex cholinergic innervation in this type of learning. (Supported by the Department of Veterans Affairs.)

760.3

INTRASEPTAL INJECTION OF 192 IgG SAPORIN, AN IMMUNOTOXIN, REDUCES LATENT INHIBITION IN A CONDITIONED TASTE AVERSION PARADIGM. K.D. Dougherty*, Salat, D.H., Walsh, T.J. Rutgers University, New Brunswick,NJ, 08903,

When animals are pre-exposed to a CS prior to its pairing with a US, the acquisition of the conditioned response is impaired. This phenomenon is called latent inhibition (LI) and is often used as a measure of selective attention. The role of cholinergic mechanisms in selective attention was assessed using a conditioned taste aversion/ LI paradigm. 192 IgG saporin (SAP) (375 or 100 ng/0.45 ul) or artificial cerbrospinal fluid (aCSF) (0.45ul) was injected into the medial septal area of male Sprague Dawley rats. aCSF subjects given 4 pre-exposures to a novel saccarhin (0.2%) solution prior to its pairing with lithium chloride (0.15M) later displayed robust LI during a two bottle choice test. In contrast, subjects treated with either 100 or 375 ng SAP displayed significantly attentuated LI. Nonpre-exposed SAP treated rats displayed aversions to saccarhin rates of extinction comparable to those of controls. Both 100 and 375 ng SAP significantly decreased high affinity choline uptake (HAChU) in the hippocampus and cingulate cortex. Only the 375 ng dose caused significant HAChU decreases in entorhinal cortex. HAChU was not altered in striatum. HPLC analysis performed in a subset of subjects revealed no effect of SAP on regional concentrations of norepinephrine, serotonin, dopamine or their metabolites in hippocampus. The results indicate that there may be considerable overlap between the cholinergic systems that mediate working memory and those that are critical to selective attention.

760.4

Effects of 192 IgG Saporin Induced Lesions of the Medial Septum on Two Allocentric Spatial Memory Tasks. **L.S. Janis*, Z. Fülöp, and D.G. Stein. Brain Research Lab, Inst of Animal Behav, Dept of Psychology, Rutgers University, Newark, New Jersey, 07102.**

Previous studies have suggested that the hippocampal system is important for processing allocentric spatial information and that damage to the medial septum (MS) impairs an animal from using allocentric information to solve a spatial task. Because the cholinergic input to the hippocampus via the MS is necessary to maintain normal electrical activity and normal physiological functions of the hippocampus, we sought to determine whether hippocampal allocentric spatial ability was dependent upon this input. Twenty male rats were initially trained on a win-shift radial arm maze (RAM) task and then given either sham or cholinergic induced (192 saporin) lesions of the MS. Rats were then tested postoperatively on the RAM to assess their allocentric spatial ability. The results indicated that while rats with cholinergic MS lesions were significantly impaired in resolving the task, they did not exhibit deficits in allocentric spatial ability. In addition to RAM testing, rats were also tested on a modified water maze task designed specifically to test allocentric spatial ability. In this task, rats with cholinergic MS lesions performed as well as sham rats. Taken together these results suggest that the cholinergic projection from the MS is not critical for normal allocentric spatial ability and that such ability can be maintained by the remaining septohippocampal input.

Supported by GenRe Corp. and Sigma Xi student research grant.

760.5

LATERAL ENTORHINAL CORTEX ASPIRATION IMPAIRS ASSOCIATIVE "ODOR-PLACE" LEARNING IN RATS. T. Otto*, K.M. Schiller, G. Cousens, & C. Ding. Program in Biopsychology and Behavioral Neuroscience, Dept.of Psychology, Rutgers University, New Brunswick, NJ 08903.

Neuropsychological, anatomical, and electrophysiological data indicate that the lateral entorhinal cortex (LEC) is an important area of convergence of olfactory information and hippocampal processing during learning. In an attempt to explore the potential functional role of the LEC in the acquisition of the relations between olfactory and spatial stimuli, we have recently developed an "odor-place" task in which rats are required to learn to go to one location in the presence of a particular odor and to a second location in the presence of a different odor in order to obtain water reinforcers. Thus, accurate performance in this task requires learning the relationship between a specific odorant and a discrete spatial location. Bilateral aspiration of the LEC resulted in a marked deficit in acquisition of this task relative to sham-operated subjects; control experiments indicate that this impairment is not due to a sensory deficit. These data are consistent with the notion that the LEC participates critically in the acquisition of associations between multiple, discrete stimuli.

760.6

THE ROLE OF THE MEDIAL SEPTAL AREA AND THE PRELIMBIC CORTEX IN MEDIATING PERFORMANCE IN AN OPERANT WORKING MEMORY TASK. J.M. Williams*, E. Mohler, A. Canacci & B.S. Givens. Dept. of Psych.., The Ohio State Univ., Columbus, OH 43210.

Both the medial septal area and the prelimbic area of the prefrontal cortex have been implicated in working memory. However, it is not clear whether deficits following damage to the medial septal area and prelimbic cortex are due to memory per se or to attentional/encoding processes. To examine the nature of septal and prelimbic deficits, rats were trained on an operant task that allowed for separate analysis of the attentional and working memory components. Rats (divided into DMTS and DNMTS groups) were tested in an operant chamber with three lights located on the front panel. Levers were located beneath each light. A tone signalled the start of the sample phase of each trial. In the sample phase, rats were presented with either a signal in which the center light was illuminated or no signal. To assess the attentional components of the task, signal length was varied (one or two seconds). After the sample stimulus, a delay of 0 or 2 seconds was introduced. This delay constituted the working memory component of the task. During the choice phase, a light appeared randomly over either the right or left lever. The opposite side remained unlit. In order to obtain a subsequent reward rats were required to press the lever that either matched (DMTS) or did not match (DNMTS) the sample phase stimulus.

After reaching criterion, reversible inactivation (tetracaine infusions) and reversible manipulations of the GABAergic and cholinergic systems (muscimol, scopolamine and carbachol infusions) of each of these areas were performed to determine the extent and nature of medial septal and prelimbic involvement in attention and working memory. Preliminary results suggest that infusions into both the medial septal area and the prelimbic cortex produce delay-dependent deficits in performance of this visual working memory task.

760.7

EFFECTS OF PERIRHINAL OR RHINAL CORTEX LESIONS ON OBJECT-MEMORY TASKS IN THE RAT. T.J. Kornecook*, M. Liu, C.A. Duva, A. Anzarut, and J.P.J. Pinel. Dept. Psychol., University of British Columbia, Vancouver, B.C., Canada. V6T 1Z4.

Work with both monkeys and rats has shown that damage limited to the rhinal cortical region (entorhinal cortex + perirhinal cortex) is sufficient to produce severe impairments in object-recognition memory (Murray et al, 1989; Mumby et al., 1994). In primates, this impairment is largely due to the perirhinal component of the lesion, as ablations of the perirhinal cortex alone produces DNMS deficits nearly as severe as those seen after complete rhinal cortex lesions, whereas ablations of the entorhinal cortex produce only a mild deficit (Meunier et al., 1993). To assess the contribution of the perirhinal cortex to object memory in rats, we compared the performance of animals with lesions limited to this area to that of animals with combined perirhinal and entorhinal cortex ablation on a rodent version of DNMS, as well as on three different object-memory tasks which resemble those used to study amnesia in humans and monkeys: (1) object discrimination, (2) discrimination reversal, and (3) 8-pair concurrent object discrimination learning.

Male Long-Evans rats received either bilateral aspiration lesions of the perirhinal cortex alone (Per), perirhinal + entorhinal cortex aspirations (Rh), or sham sugery (Cont). The Rh group was significantly impaired with respect to the Cont group on postsurgery DNMS testing at delays of 60 sec($p<0.05$), and impaired with respect to both control and perirhinal-lesioned rats at delays of 120 sec ($p<0.01$). Although the DNMS scores of the Per group were lower than the controls at almost all delays, no statistical differences were found. On the simple object discrimination task, both the Per and Rh groups were equally impaired relative to the control rats ($p<0.02$). There were no statistical differences, however, in the rate at which all three groups acquired the discrimination reversal. With respect to the concurrent object discrimination task, a significant impairment arose only when the Per and Rh animals were combined into a single, uniform group ($p<0.03$). These results provide further evidence for the importance of the rhinal cortex in object-memory processes in the rat.

760.8

THE EFFECTS OF SEPARATE AND COMBINED LESIONS OF THE PERIRHINAL CORTEX, FORNIX AND SEPTUM ON MEMORY IN THE RAT. K. A. Wiig, S. J. Booth, P. Liu, L. Cooper*, and D. K. Bilkey. Department of Psychology, University of Otago, Dunedin, New Zealand.

Sham operated control rats and rats with bilateral lesions of either the perirhinal cortex, fimbria-fornix, or both these structures, were tested on a two choice discrimination task and an object-guided DNMS task (Mumby et al, 1990). All lesioned rats were able to learn the discrimination task at a rate comparable to control animals. In contrast, animals with lesions of the perirhinal cortex or fimbria-fornix exhibited a moderate, delay dependent memory deficit on the DNMS task, as they performed normally at short delay intervals, but were impaired relative to control rats at delay intervals of 30 seconds or more. Animals with combined lesions displayed a significantly greater impairment on the DNMS task than animals with lesions of either structure alone. The combined lesioned rats were severely impaired in acquisition of the task, and displayed a profound memory deficit at delay intervals of 15 seconds or more. We are currently investigating the effects of transient lesions of either the perirhinal cortex, medial septum or combined lesions of both these regions on watermaze performance. Preliminary data suggests that combined but not separate lesions produce a marked disruption in spatial memory. Overall, these results suggest that the perirhinal cortex and septohippocampal system may function synergistically in both spatial and nonspatial memory processes.

Supported by the Health Research Council of New Zealand.

760.9

PERIRHINAL CORTEX DAMAGE: EFFECTS ON ACQUISITION AND RETENTION OF OBJECT AND PLACE DISCRIMINATIONS IN RATS. R.S. Astur*, D.G. Mumby, and R.J. Sutherland. Depts. of Psychology and Physiology, Univ. of New Mexico, Albuquerque, NM 87131.

In order to examine the effects of perirhinal cortex lesions on object memory, 12 rats were trained on 5 object discriminations 1, 4, 7, 10, and 13 week(s) prior to surgery. To examine place memory, each rat was trained in 3 different water task problems 2, 8, and 14 weeks prior to surgery. Six rats then received bilateral aspiration lesions of perirhinal cortex, and 6 rats received sham lesions. Retrograde effects were assessed by testing all rats on the five object discriminations and the three pool problems that they had learned previously. Anterograde effects were assessed by teaching the rats two new object discriminations and one new pool problem. For the old object discriminations, there were no differences between the groups in relearning the discriminations nor in their accuracy in the first five trials of testing. For the new object discriminations, there were no significant differences between the groups in learning the new object discriminations. For the old pool problems, there were no differences between groups in percent of time spent in the correct quadrant during probe trials or first-trial escape latency, although there was a trend for the perirhinal group to be impaired on these measures. These data suggest that perirhinal cortex plays a nonessential role in the acquisition and retention of object and place discriminations.

760.10

POSTTRAINING LESIONS OF PERIRHINAL CORTEX DECREASE PASSIVE AVOIDANCE OF PUNISHED DRINKING BUT NOT ITS REINSTATEMENT. G.D. Coover* and S.J. Mertes. Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

Lesions of the rostral perirhinal cortex (rPRh) of the rat disrupt fear conditioned to discrete stimuli (Rosen et al., J. Neurosci. 12:4624-4633, 1992) and also contextual stimuli (Corodimas and LeDoux, Soc. Neurosci. Abstr. 20:1007, 1994). The present study examined whether rPRh lesions would disrupt the context-dependent memories of previously-learned passive avoidance of drinking (dPA).

Male Long-Evans rats were water-deprived and trained to a criterion of 5 min avoidance of drinking by punishing drinking with .5-sec footshocks (FS) that increased in intensity over trials. They learned within 11 - 20 FS (Mdn = 16, M = 15.9, SD = 2.6). Five days later they received either control surgery or 1-mA/15-sec anodal lesions of rPRh or the rostral central nucleus of the amygdala (rACe). Two weeks later latency to drink without further FS was assessed in the testing chamber. They were allowed to drink for 30 sec. Rats not returning to drink within 5 min were given another session the next day. All rats were given two additional, daily sessions of 1-min cumulative drinking and then tested for reacquisition of dPA.

When tested two weeks after surgery, the 8 control rats took at least 168 sec to drink (Max = 788, M±SE = 373±90). All 9 rPRh rats drank in less than 95 sec (Min = 34, M±SE = 61±7). The rACe group also returned sooner than the control group (Range 50 - 220, M±SE = 114±27). Despite the apparently greater retention of dPA by controls, they did not reacquire dPA with fewer FS trials (M±SE = 3.25±0.49) than the rPRh group (M±SE = 3.67±0.24). The rACe group exhibited a major deficit in dPA by requiring 32.0±3.6 FS to avoid for 5 min.

In reacquisition, the rPRh rats actively avoided the spout after the 1st FS (3 rats) or 2nd FS (the other 6) and they exhibited approach/avoidance of the spout on that trial (6 rats) or after one more FS (the other 3). The lesions appeared to alter the "state" in which memory of the punishment contingency was being retrieved.

760.11

AMYGDALA $GABA_A$ RECEPTORS MODULATE RETENTION OF CHANGES IN REWARD MAGNITUDE. J.A. Salinas* & J.L. McGaugh. Center for the Neurobio. of Learning & Memory and Dept. of Psychobio., U. of Calif., Irvine, CA 92717-3800.

We have previously shown that post-training amygdala inactivation impairs memory for reward reduction. In the present study we infused a $GABA_A$ agonist (muscimol, MUS) or antagonist (bicuculline methiodide, BMI) into the amygdala immediately after a reward shift. Rats with bilateral amygdala cannulae were trained to run a straight alley for a large or small food reward. In Experiment 1 rats in the large-reward group were shifted to the small reward and received an infusion of vehicle or MUS bilaterally into the amygdala immediately after. Shifted vehicle animals displayed an increase in runway latencies compared to unshifted controls. In contrast, shifted MUS animals displayed latencies comparable to unshifted animals by the second postshift day. These findings suggest that muscimol attenuated the memory of the reward reduction. In Experiment 2 rats were trained as before except they received BMI immediately after reward reduction. Shifted BMI animals displayed elevated runway latencies compared to shifted vehicle animals by the second postshift day. These findings suggest that BMI enhanced memory of the reward reduction. In Experiment 3, animals were trained as before except they first experienced a reward increase before receiving post-training injections of vehicle, MUS or BMI. On the next day the reward was reduced. Despite reward reduction, shifted BMI animals persisted displaying low latencies for more trials than shifted MUS animals. These findings suggest that BMI enhanced memory for the reward increase. Further, the findings suggest that the amygdala and its GABAergic system is involved in memory consolidation for both positive and negative affective experiences.

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760.12

BASOLATERAL AMYGDALA LESIONS BLOCK GLUCOCORTICOID-INDUCED MODULATION OF MEMORY FOR SPATIAL LEARNING. B. Roozendaal*, G. Portillo-Marquez and J.L. McGaugh. Center for the Neurobiology of Learning and Memory, and Dept. of Psychobiology, Univ. of California, Irvine CA 92717-3800.

This study examined the involvement of the amygdala in the effects of glucocorticoids on the formation of memory for spatial learning in a water maze. Male Spraque-Dawley rats were given five training trials of spatial orientation learning in a water maze, and retention was tested 48 h later. Removal of the adrenal glands (adrenalectomy; ADX) 4-5 days prior to training significantly impaired memory for this task, and immediate posttraining systemic (s.c.) injections of dexamethasone (0.3 mg/kg), a potent synthetic glucocorticoid, attenuated the memory impairment. In contrast, injections of corticosterone (0.3 mg/kg) were ineffective. Neurochemically-induced lesions of the basolateral (BLA), but not of the central (CEA) or medial (MEA) nucleus of the amygdala blocked the modulatory effects of short-term ADX and dexamethasone administration on spatial memory. These findings are consistent with previous evidence that the dexamethasone-induced enhancement of memory for inhibitory avoidance training was also selectively blocked by lesions of the BLA. ADX also impaired acquisition performance in the water maze. Lesions of the CEA, MEA, as well as lesions of the BLA, appeared to block the impairing effects of ADX on acquisition performance in the water maze. Acquisition and retention performance were not significantly affected by BLA lesions in otherwise untreated animals. In contrast, lesions of the CEA resulted in both deficient acquisition and retention performances, and lesions of the MEA resulted in an impaired retention. These experiments further provide evidence that the BLA is a critical area involved in integrating hormonal influences on learning and memory.

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760.13

STRIA TERMINALIS LESIONS DO NOT BLOCK BENZODIAZEPINE-INDUCED ANTEROGRADE AMNESIA. O. Carmi* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory, and Department of Psychobiology, University of California, Irvine, CA 92717-3800.

Extensive evidence indicates that benzodiazepines (BZ) induce anterograde amnesia when administered systemically or directly into the amygdala before training. Furthermore, lesions of the amygdala block the BZ-induced anterograde amnesia. The stria terminalis (ST) is a major input/output pathway of the amygdala. ST lesions, similar to amygdala lesions, have been shown to block the memory enhancement produced by hormonal and neurotransmitters systems, including adrenergic, cholinergic, opioid peptidergic and glucocorticoid systems. Such findings imply that ST lesions should also block BZ effects on memory. The present study examined this hypothesis in experiments using two tasks, inhibitory avoidance and spatial orientation water maze tasks. Bilateral ST lesions were produced by radio-frequency current in male Sprague-Dawley rats and one week later, ten minutes prior to inhibitory avoidance training (0.45mA, 1s), rats received injections of saline or the BZ agonist midazolam (MDL, 1 mg/kg, i.p.). On a 48-h retention test, the retention latencies of MDL-treated rats were lower than those of saline-treated rats. This BZ-induced retention impairment was not blocked by ST lesions. For the water maze experiment, rats pretreated with MDL, received five training trials to find the hidden platform. The MDL impaired acquisition. Furthermore, this BZ-induced acquisition effect was not blocked by ST lesions. On a 48-h retention test, MDL-treated rats were still impaired relative to their control group. These data suggest that the BZ-induced memory effects are not dependent upon intact ST. These data conflict with those of many previous studies examining drug effects on learning and memory on animals with ST lesions. A possible explanation for this discrepancy is that BZ-induced amnesia is may be due to an impairment in acquisition rather than modulation of memory storage.

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760.14

LARGE UNILATERAL LESIONS OF THE AMYGDALA AND MEDIAL PREFRONTAL CORTEX ON OPPOSITE SIDES OF THE BRAIN DO NOT PREVENT ACQUISITION OF CONDITIONED BRADYCARDIA. Mark Chachich*, D.A. Powell, James Penney, Saera Saleem & Mike Kaczmarek. VA Medical Center and University of South Carolina, Columbia, SC 29208.

We reported at last year's meeting that unilateral lesions of the central nucleus of the amygdala (ACN) and medial prefrontal cortex (mPFC) on opposite sides of the brain diminished but did not prevent acquisition of conditioned bradycardia. These data seemed to suggest separate and parallel control of conditioned bradycardia by the amygdala and mPFC. However, connections between mPFC and the amygdala originate not in the ACN (except for its most ventral aspects) but in the basolateral (BL) and lateral (L) nuclei. Thus, in the present experiment unilateral lesions were made on opposite sides of the brain centered on the mPFC and the BL and L nuclei of the amygdala. One session of differential heart rate (HR) conditioning was administered, in which tones served as CSs and paraorbital shock served as USs. Although large bilateral lesions of the amygdala completely abolished conditioned bradycardia, unilateral lesions of the mPFC and amygdala on opposite sides of the brain again diminished but did not prevent occurrence of the decelerative HR conditioned response.

Supported by VA Institutional Research Funds

760.15

EXCITOTOXIC LESIONS IN THE BASOLATERAL AMYGDALA SELECTIVELY ABOLISH DELAYED CONDITIONED TASTE AVERSIONS IN RATS. M. Foy[1]*, S. Maren[2] and D. Mitchell[3]. [1]Dept. of Psychol., Loyola Marymount Univ., Los Angeles, CA 90045, [2]Dept. of Psychol., Univ. of California, Los Angeles, CA 90024, [3]Dept. of Psychol., Univ. of Southern California, Los Angeles, CA 90089.

The amygdala mediates a variety of associative and nonassociative learning processes, including conditioned taste aversions (CTAs) and taste neophobia. Previous research has shown that electrolytic lesions in the amygdala disrupt immediate CTAs in which illness immediately follows consumption of a novel solution, but that excitotoxic lesions do not. However, the effects of amygdala lesions on delayed CTAs in which illness follows consumption of a novel solution by several hours have not been investigated. Given that long delay learning is a salient characteristic of CTAs and theoretical assertions that delayed CTAs are mediated by nonassociative processes (Mitchell, Scott & Mitchell, 1977), we reasoned that delayed CTAs would be especially susceptible to amygdala damage. Accordingly, excitotoxic lesions were made in the basolateral amygdala of pentobarbital-anesthetized male rats using *N*-methyl-D-aspartate; control rats received sham surgery with no drug infusion. One week following recovery from surgery, half of the rats from each group were made ill with LiCl injections administered either immediately or 4 hours after consumption of a novel saccharin solution. Neophobia controls were surgically treated as above, but received no saccharin or LiCl treatment. All groups were subsequently administered a two-bottle preference test (water vs. saccharin) during a 20 day period. The results showed that basolateral amygdala lesions blocked both taste neophobia and delayed CTAs, but had no effect on immediate CTAs. These data demonstrate that taste neophobia and delayed CTAs share a common nonassociative process that requires neurons in the basolateral amygdala, and support the theory that immediate and delayed CTAs are differentially mediated by associative and nonassociative learning processes.

760.16

INVOLVEMENT OF THE PERIAQUEDUCTAL GRAY IN FEAR-POTENTIATED STARTLE. **D.L. Walker* and M. Davis. Dept. of Psychiatry, Yale University Sch. of Med., New Haven, CT 06508.**

The acoustic startle response is reliably enhanced when elicited in the presence of a cue previously paired with moderately intense footshock (i.e., fear-potentiated startle). Interestingly, training with higher intensity footshocks produces relatively poor potentiated-startle. Because recent evidence suggests that particularly aversive stimuli may activate the dorsolateral periaqueductal gray (dl-PAG) and that behaviors associated with dl-PAG activation may be incompatible with startle, we hypothesized that stimuli paired with high-intensity footshock might also activate this system and interfere with the expression of fear-potentiated startle. To test this hypothesis, two experiments were conducted.

In Experiment I, rats received 30 light-shock pairings using either moderate (0.6 mA) or high (1.6 mA) footshocks, followed by excitotoxic lesions of the dl-PAG or sham lesions. When trained at 0.6 mA, fear-potentiated startle was comparable in both sham and lesioned animals. When trained at 1.6 mA, however, potentiated startle was reliable only in lesioned rats. Baseline startle was unaffected. In Experiment II, rats trained at 0.6 mA were tested immediately after intra-PAG infusion of either distilled water or an excitatory non-toxic dose of kainic acid. Kainic acid infusions significantly disrupted fear-potentiated startle but did not affect baseline startle.

Together, these results suggest that the dl-PAG may be activated by stimuli associated with particularly aversive events and that this activation may interfere with the expression of fear-potentiated startle.

760.17

THE EFFECTS OF OLFACTORY BULBECTOMY ON THE ACOUSTIC STARTLE REFLEX AND ITS MODULATION BY STRESS. K.A. McNish* and M. Davis. Depts. of Psychiatry and Psychology, Yale Univ. Sch. of Med., New Haven, CT 06508.

The effects of olfactory bulbectomy on the acoustic startle reflex, fear-potentiated startle, and shock-induced sensitization of the acoustic startle reflex were examined in a series of three experiments. In Experiment 1, bilateral olfactory bulbectomy resulted in an increase in the acoustically elicited startle reflex relative to sham operated controls. The increase in baseline startle was persistent for up to five weeks following the lesion. Bulbectomized animals showed normal acquisition of fear-potentiated startle but enhanced context conditioning relative to sham operated controls. In Experiment 2, olfactory bulbectomy resulted in an increased sensitivity to footshock-induced sensitization (0.6 mA and 1.0 mA) of the acoustic startle reflex relative to sham and unoperated controls. In Experiment 3, bulbectomized animals showed shock sensitization to a shock level (0.3 mA) which did not produce sensitization in sham and unoperated controls. Taken together, these data suggest that olfactory bulbectomy results in an increased vulnerability to stressors, perhaps because of a disinhibition of the amygdala. Thus, the olfactory bulbectomy model of depression may share some similarities with other stress-induced models of depression.

760.18

MEDIAL PREFRONTAL CORTEX (mPFC) LESIONS FOLLOWING FEAR CONDITIONING DO NOT RETARD EXTINCTION OF THE FEAR-POTENTIATED STARTLE RESPONSE. J.C. Gewirtz* & M. Davis, Depts. of Psychiatry and Psychology, Yale University Sch. of Med., New Haven, CT 06508.

Although the neural systems involved in fear conditioning are relatively well elucidated, much less is known about the neural systems involved in extinction of conditioned fear. Recently, it was shown that mPFC lesions made before acquisition of conditioned fear retarded subsequent extinction of conditioned freezing (Morgan, Romanski & LeDoux, 1993). The present study examined the effects of lesions of mPFC on extinction of another conditioned response, fear-potentiated startle, when the lesions were made after acquisition of conditioned fear.

In each of 12 acquisition sessions, rats were given two presentations of a 3.7-s light CS which terminated with a 0.5-s, 0.6-mA footshock US. To assess fear-potentiated startle, each session also included presentations of a 50-ms, 95-dB startle-eliciting stimulus alone, and of the same stimulus in the presence of the visual CS. Rats showed a gradual increase in startle to the startle-eliciting stimulus alone (contextual conditioning) and an additional gradual increase in startle to the same stimulus presented in the presence of the light (explicit cue conditioning). On the basis of their terminal levels of fear-potentiated startle, rats were matched into two groups. One group received electrolytic lesions of mPFC (n=24) and the other group received sham lesions (n=20). Both groups received 18 extinction sessions, starting one week after surgery. These were identical to acquisition sessions, except that the US was no longer presented. Both groups showed gradual extinction of fear-potentiated startle to the explicit cue and to the experimental context. In addition, they showed equivalent "reinstatement" of fear-potentiated startle when retested one day after receiving 5 presentations of the US alone. Hence, under these conditions, lesions of the mPFC did not have any effect on extinction or reinstatement of conditioned fear.

Experiments are currently under way to evaluate the degree to which the role of the mPFC in extinction depends upon the level of initial excitatory conditioning, the modality of the CS, the nature of the conditioned response, and when lesions are made relative to the time of excitatory conditioning.

760.19

LESIONS OF THE PERIRHINAL CORTEX BLOCK CONDITIONED EXCITATION BUT NOT CONDITIONED INHIBITION OF FEAR MEASURED WITH FEAR-POTENTIATED STARTLE. W.A. Falls* , K. Bakken, S. Webster & M. Davis, Dept. Psychology, Northern Illinois University, DeKalb, IL 60115 and Dept. of Psychiatry, Yale Univ., Med. Sch., New Haven, CT 06508.

Although much is known about the neural systems responsible for the acquisition and expression of conditioned fear, little is known about the neural systems responsible for the inhibition of fear. Following a conditioned inhibition procedure in which a light is repeatedly paired with shock (i.e., light+) and a light-noise compound is presented without shock (i.e., noise→light-), the noise acquires the ability to inhibit fear-potentiated startle to the light in a noise→light summation test (Falls & Davis, 1993; *Neurosci. Abs.* vol. 19, p.372, #155.6).

Perirhinal cortex lesions performed after light+shock training block fear-potentiated startle. However, fear-potentiated startle to the light can be re-acquired. Because of this, it is possible to use a summation test to assess whether lesions of the perirhinal cortex will disrupt previously acquired conditioned inhibition. Rats were given 15 light+shock pairings on each of 2 days followed by conditioned inhibition training consisting of 5 light+ trials intermixed with 15 noise→light- trials on each of 5 days. Testing occurred 1 day later. All rats showed conditioned fear to the light as defined by greater startle amplitude in the presence of the light than in its absence. All rats showed conditioned inhibition of fear, defined as a reduction in fear-potentiated startle to the light when accompanied by the noise. Next, half of the rats were given perirhinal cortex lesions and half were sham operated. All rats were tested 1 week later. As expected, lesions of the perirhinal cortex blocked fear-potentiated startle to the light. On each of the next 5 days, the rats were retrained with light+ trials with no further conditioned inhibition training. Testing occurred 1 day later. Perirhinal cortex lesioned rats re-acquired fear-potentiated startle to the light. Importantly, the noise conditioned inhibitor retained its ability to inhibit fear-potentiated startle to the retrained light.

Although the perirhinal cortex is critical for initial performance of fear-potentiated startle, it is not critical for the expression of conditioned inhibition. We have reported that lesions of the central nucleus of the amygdala also fail to prevent the expression of conditioned inhibition to a retrained light (Falls & Davis, *Behav. Neurosci*, 109(3), 379-387). Hence, inhibition of fear-potentiated startle is likely to occur elsewhere in the fear-potentiated startle pathway.

760.20

THE EFFECTS OF EXCITOTOXIC LESIONS OF PIRIFORM CORTEX, ENTORHINAL CORTEX, AND MEDIAL THALAMUS ON CONTINUOUS OLFACTORY NON-MATCHING TO SAMPLE (CONMTS) AND DISCRIMINATION Y.P. Zhang* and , R. G. Mair. Dept. of Psychology, Univ. of New Hampshire, Durham, NH 03824.

Forty-eight male rats were pre-trained on a go/ no-go CONMTS task. Rats were matched for performance and randomly assigned to one of the following treatment groups (N=8 in each group); excitotoxic lesions of piriform cortex (PIR), entorhinal cortex (ENT), lateral internal medullary lamina of thalamus (LIML), medial dorsal nucleus of thalamus (MDn), nonspecific nuclei of thalamus (NONS), and sham controls (CON). After recovery from surgery, the rats were retrained on CONMTS and then on a go/no go discrimination trained with procedures matched to CONMTS.

Initial analyses indicate that only the PIR and LIML groups were significantly impaired on CONMTS. The ENT and MDn groups showed transient impairments like those found in an earlier study for frontal cortical lesions. All groups learned the discrimination task a normal rates. These results are consistent with the hypothesis that olfactory pathways beyond piriform cortex are necessary for olfactory memory, but not discrimination.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS XII

761.1

Disinhibition of reinforcing brain stimulation following lesions in the region of the tuberomammillary nucleus and the CA1 region of the hippocampus

Wagner, U., Zimmermann, P. and Huston, J.P., Institute of Physiological Psychology, University of Düsseldorf, 40225 Düsseldorf, Germany (EBBS)

The subnuclei of the tuberomammillary nucleus (TM) are located in the posterior part of the hypothalamus.The neurons of this nucleus innervate extensive parts of the brain with several transmitters and they represent the only source of histaminergic projections in the brain. Studies are presented dealing with lateral hypothalamic self-stimulation behavior in rats following unilateral lesions in this region and in the hippocampus, which has been described as the only ipsilateral projection area of the TM.

A unilateral electrolytic lesion of the TM led to an ipsilateral increase in self-stimulation behavior beginning with the first post-lesion day. Response rates continued to rise for two weeks post-lesion. In a follow-up study the effects on self-stimulation were replicated by lesioning the TM with ibotenic acid, indicating that the observed disinhibitory effects in both experiments are based on the destruction of intrinsic cells and not on fibers passing through the lesioned area. A projection area possibly involved in the observed lateralized amplification of brain stimulation reward is the CA1 region of the hippocampus. Following a unilateral ibotenic acid lesion in this structure we observed an increase in bar pressing on the contralateral side of the lesion. The lesion did not result in a greater resistence to extinction; thus, it is likely that the increase in response rates can be interpreted in terms of an amplification of the rewarding value of brain stimulation and not in terms of a response perseveration which has been described as a result of hippocampal ablation.

Our results suggest that the TM together with the hippocampus exerts an inhibitory control over lateral hypothalamic self-stimulation. These results are the first to focus on an inhibitory element in the neural substrate of reinforcement.

761.2

MEMORY ENHANCING EFFECTS OF IBOTENIC ACID LESIONS OF THE TUBEROMAMMILLARY NUCLEUS AND EFFECTS ON BRAIN HISTAMINE. J.P. Huston[1]*, Ch. Frisch[1], H.W.M. Steinbusch[2], R.U. Hasenöhrl[1]. European Graduate School for Neuroscience i.f. 'Brain and Behavior'. [1]Inst. Physiol. Psychology, Univ. Düsseldorf, FRG.; [2]Dept. Psychiatry and Neuropsychol., Univ. Limburg, Maastricht, The Netherlands.

The tuberomammillary nuclei (TM) are located in the posterior part of the hypothalamus and provide the main source of neural histamine (HA) in the brain. Our experiments performed with bilateral lesions of the TM region revealed a role of the TM system in learning and memory processes. These lesions facilitated inhibitory avoidance performance and improved long-term retention in a spatial discrimination test in both adult and aged rats. However, since dc-lesions were performed, it was not possible to point at the TM neurons as responsible for the observed effects on learning and memory. Thus, the objectives of a follow-up study were two-fold. In order to determine whether the facilitation of learning was due to the destruction of intrinsic TM neurons a bilateral ibotenic acid lesion of the TM was performed and the rats were tested along with sham-lesioned controls on different versions of the Morris water maze task. In order to determine whether the behavioral effects revealed upon destruction of the TM neurons involve histaminergic mechanisms concentrations of HA and HDC were determined in different brain regions using immunohistochemical methods. The main finding of this study was that rats with neurotoxic lesions of the TM showed an accelerated acquisition rate in the course of place learning and an improved ability to locate the platform site during a spatial probe trial. Furthermore, following destruction of the TM region changes in HA and HDC concentrations were observed in several brain regions, including the site of lesion in the TM, basal forebrain nuclei and hippocampus. These results indicate that the facilitatory effects on learning following TM lesion are based on the destruction of intrinsic TM neurons and suggest that this facilitation may be related to the function of the histamine system.

761.3

SELECTIVITY OF SPATIAL LEARNING IMPAIRMENT IN RATS WITH LESIONS OF THE MAMMILLARY REGION V. Sziklas*, M. Petrides, and P. Jackson. McGill University, Montreal, Quebec, Canada

Rats with lesions of the mammillary region (MB-R), the dorsal hippocampus (H), or a control operation (OC) were trained on a spatial conditional associative learning task in which they had to learn to respond to one of two visual stimuli depending on their location in an open field. In a second study, rats with similar lesions were trained on a place learning task which required them to locate a hidden platform in a pool of opaque water. Rats with damage to the MB-R were able to acquire the conditional associative task at a rate comparable to that of the OC animals. The H group was significantly impaired in comparison with both the MB-R and the OC groups. By contrast, both the MB-R and the H animals were slower to acquire the location of the hidden platform in comparison with the OC group. Taken together with earlier findings from our laboratory, the present results suggest that damage to the MB-R impairs the ability to learn and remember the location of spatial cues but does not affect the capacity to discriminate between particular visual stimuli, depending on the location within which they are embedded.

761.4

ASSESSING THE CONTRIBUTIONS OF DIFFERENT ANTERIOR THALAMIC NUCLEI TO SPATIAL MEMORY J.P. Aggleton*, S. Nagle, N. Neave and P. Hunt. School of Psychology, University of Wales, Cardiff, CF1 3YG .

Rats were prepared with neurotoxic lesions centred in either the anterior medial (AM, n= 8) or the anterior ventral (AV, n=8) thalamic nuclei. These were compared with a further group of rats with neurotoxic lesions in both nuclei (AM+AV, n=7) and a group of surgical controls (SHAM, n=11). The lesions were produced by injecting N methyl-d-aspartate acid (0.12M). Following recovery from surgery the rats received 16 sessions (6 trials/session) of a food rewarded forced-alternation task in a T-maze. The AM+AV group were severely impaired throughout this training, but the AM and the AV groups displayed equivalent, mild impairments. The AM and AV groups were able to match the SHAM animals by the end of training. A series of sessions using a cross-shaped maze then confirmed that the SHAM, AV, and AM animals were able to use allocentric cues. The four groups were then tested in the cross-maze on an egocentric discrimination and single reversal (always turn in one direction, then after reaching criterion always turn the other way). None of the groups were impaired, the AM+AV animals showing a slight advantage. Finally, the animals were tested on the radial arm maze, where only the AM+AV group showed a clear impairment. The results help to confirm the importance of these nuclei for allocentric memory tasks, but indicate that neither nucleus is pre-eminently important.

761.5

AUDITORY DELAYED RESPONSE TASK DEFICITS FOLLOWING MEDIAL THALAMIC AND PREFRONTAL CORTICAL LESIONS IN THE RAT. A.A. Stevens, R. G. Mair*. Dept. of Psychology, Univ. of New Hampshire, Durham, NH 03824.

To study auditory memory in a rodent model of Korsakoff's syndrome, 24 male rats were pre-trained on an auditory conditional discrimination task which required the rat to respond to one location following a 3 kHz tone and a second location following an 11 kHz tone. Retention intervals (RI) were imposed by blocking access to the response locations with a gate. Treatments included sham control (CON, n=8) and lesions of medial wall of frontal cortex (MW, n=8) or the lateral internal medullary lamina of thalamus (LIML, n=8). Throughout extensive post-surgical training, the LIML group showed a chronic and significant deficit even when the tones remained on until a response was made. The MW group also performed worse than the CON group when no RI was instituted but not significantly so. Both lesion groups also performed poorly across retention intervals from 0 - 6.4 sec, although there was no evidence of abnormally rapid forgetting. These results suggest that medial thalamic and frontal cortical lesions can produce a conditional discrimination deficit even when there is no memory requirement. Because the LIML group was also impaired on a simple auditory discrimination, it is not certain whether this deficit is the result of an impaired ability to discriminate pure tone stimuli.

761.6

VALIDATING THE DIRECT METHOD FOR MEASURING THE SUBJECTIVE MAGNITUDE OF BRAIN STIMULATION REWARD. M. I. Leon* & C. R. Gallistel. Dept. of Psychology, UCLA, Los Angeles, CA 90095-1563.

The direct method for scaling subjective reward magnitude as a function of stimulation strength uses a two-lever choice paradigm with concurrent variable interval schedules of reward. The rat allocates its time between a "standard" lever delivering rewards of fixed magnitude, and an "alternate" lever delivering rewards that vary in magnitude over trials. The validity of the procedure rests on the assumption that subjective reward magnitudes and relative rates of reward combine multiplicatively to determine time allocation. If this assumption is correct, a rat will equiprefer a particular reward being delivered every ten seconds to a reward half that size being delivered every five seconds. This experiment was designed to test this assumption. The results in self-stimulating rats (male Sprague-Dawley) with electrodes in the lateral hypothalamus and VTA suggest that subjective reward magnitude and rate of reward combine multiplicatively when the net value of either lever does not cause the rat's time allocation between levers to differ by more than approximately 6:1. When the ratio of time distributions surpasses this limit, the necessary assumption for scaling subjective reward appears to break down. This suggests that the optimal procedure for scaling reward is to program the standard lever to deliver rewards one quarter as often as the alternate lever, and to set the reward on the standard to its maximal level. The effect of this is to prevent the net value of either lever from becoming so great that the animal's time allocation ratio differs by more than 6 to 1.

761.7

A NOVEL TWO-TRIAL MEMORY TASK WITH AUTOMATED RECORDING. INFLUENCE OF INTER-TRIAL INTERVAL INTERFERENCES AND ENVIRONMENTAL CONTEXT ON PLACE OR OBJECT RECOGNITION. F. Dellu, W. Mayo, M.P. Mano, M. Vallée, J.J. Bouyer, M. Le Moal and H. Simon*. Lab. de Psychobiologie des Comportements Adaptatifs, INSERM U.259, 33077 Bordeaux Cedex, France.

A two-trial memory task, based on place or object exploration in a Y-maze, was developed to study memory in adult and aged rats (Dellu et al., Brain Res., 588, 1992). This paradigm avoids the use of electric shocks or deprivation that may have non-specific effects, and the task does not require learning of a rule. A number of behavioral parameters in several animals could be recorded automatically.

Recognition is differently influenced both by the type of information (place vs. object) and by the inter-trial interval (recognition retention time). For place exploration, recognition was still present at the 6h inter-trial interval whereas recognition in object exploration only appeared to last for about 4h in 2-month-old rats. Memory recognition in old rats (18 months) was clearly affected by increasing the retention time. Recognition also depends on the environmental context as demonstrated by the following results : 1) place recognition remains intact in spite of an interferent exploration (one trial in another Y-maze situated in another environment) between the acquisition and the retrieval trials and 2) object recognition is impaired when the environmental context is changed between the two trials.

This is of particular interest in the light of the dual mechanism suggested to be involved in recognition i.e. determination of stimulus familiarity and stimulus identification which involves retrieval of relevant contextual information processing. It is also noteworthy that contextual information processing alterations are at the heart of age-related cognitive deficits. In conclusion this memory task combines simplicity, rapidity, sensitivity and high specificity and should be useful to neuro- and psychopharmacologists.

761.8

SAME-SESSION ANALYSIS OF WORKING AND REFERENCE MEMORY IN THE RAT: PROCEDURE. W. J. Wilson*, S. A. Bradtmiller, M. L. Felton, G. D. Lyons, L. A. Shaffer, & A. S. Zieles. Dept. of Psychological Sciences, Indiana-Purdue University, Fort Wayne, IN 46805 USA.

Eight female Sprague-Dawley rats were trained successively in two different behavioral tasks: spatial alternation and visual discrimination in a phi-maze. In spatial alternation rats were rewarded for choosing the arm not used on the previous trial. In visual discrimination rats were rewarded for selecting the lighted arm, the position of which varied randomly. Rats next experienced these two tasks on alternate days, then combined in the same session, yielding a behavioral session requiring the use of both reference (visual discrimination) and working (spatial alternation) memory. In this phase in which both tasks were employed, the rat was rewarded first for selecting the lighted arm of the maze, then for selecting the opposite arm, and so on. Thus the contingency in effect changed following each reinforcer. Each daily session lasted until the rat received 41 reinforcers (an initial free reinforcer, and 40 earned via the selection of the appropriate arm of the maze).

Rats acquired the spatial alternation task more readily than they did the visual discrimination task (means: 8.88 vs 13.00 sessions to reach the criterion of 3 successive sessions completed in fewer than 50 trials; $p < .01$), probably reflecting both the tendency to alternate spontaneously, and an initial bias to select the dark arm of the maze in the visual task. When both tasks were combined, rats performed equally well on the working and reference memory tasks in Sessions 1 - 5 (means: 23.33 [working] vs 22.63 [reference] trials to receive 20 reinforcers). Performance on the reference memory task decreased significantly by Sessions 11 - 15 (working: 22.17 vs reference: 25.23 trials, $p < .01$). This procedure will serve in future studies examining the effects of amnestic or nootropic drugs.
[Except for presenting author, order of authors is alphabetical.]

761.9

TRANSFER OF TEMPORAL DISCRIMINATION TRAINING. S.P. Clarke, R.B. Ivry, S. Roberts*, and N. Shimizu. Dept. of Psychology, University of California, Berkeley, CA 94720.

If separable neural systems exist for the perception and discrimination of different temporal intervals, then experience with one task involving discrimination between stimuli of short duration should have no effect on rate of learning upon transfer to a task requiring discrimination between stimuli of longer duration, and vice-versa. Rates of learning will be assessed for rats trained on temporal discrimination tasks in a standard operant chamber. In the first experiment, rats will be trained to discriminate between white noise stimuli in either a short range (SR; 200 vs. 800 msec) or long range (LR; 20 vs. 80 sec). A sucrose pellet is delivered for a press on one lever corresponding to the shorter stimulus in each task (200 msec or 20 sec) or for a response on the other lever for the long stimulus (800 msec or 80 sec). After training to asymptote, the two groups (SR and LR) will then be trained to asymptote on the opposite task (LR and SR) with lever mapping reversed for half of each group. If separable neural systems mediate the learning of the SR and LR tasks, then the rates of learning for all mapping groups should be the same, indicating that experience with SR timing had no effect on learning a discrimination involving LR timing, and vice-versa. The second experiment will be identical to the first, except that the groups will transferred to a medium range (MR; 2 vs. 8 sec) discrimination task. If the SR cerebellar mediated timing system is limited to perception in the milliseconds range, as our human and animal data suggest, then there should be no difference in rate of learning for the two mapping groups of rats shifted from SR to MR. Facilitation would be expected in rats shifted from LR to MR with the same mapping if a LR timing system, responsible for learning in the seconds-to-minutes range, is involved in both tasks.

761.10

HOW RATS ARE TRANSPORTED TO THE MAZE DETERMINES SUCCESS OR FAILURE OF SPATIAL PROBLEM SOLVING: ENVIRONMENTAL CUES ARE NOT SUFFICIENT TO SUPPORT SPATIAL LEARNING. G.M. Martin, C.W. Harley*, A.R. Smith, E.S. Hoyles, and C.S. Rideout. Psychology Dept., Memorial University, St. John's, NF A1B 3X9

Theories of spatial learning suggest rats possess a map based on distal environmental cues and/or environmental shape. These theories attribute spatial learning to environmental information during maze training and not to events which precede the animal's placement in the training situation.

In the present experiment two procedures were used to bring rats to a four arm elevated maze on which food was in a constant spatial location. Rats in the normal procedure were brought into the training room in their home cage and placed on a storage rack. Rats were carried from the home cage and placed on the maze on each of eight trials. Rats were returned to the home cage after a correct choice or 60 secs.

Rats in the second procedure were placed in closed metal containers, rotated six to ten times over a 60 second period and then brought into the training room and placed on the storage rack. Rats were carried in the containers to the maze, removed and placed on the maze. At the end of each trial rats were replaced in the container, returned to the storage rack and rotated. Two minutes or more intervened between rotation and subsequent maze placement. Criterion was 9 out of 10 correct.

Rats trained with the normal home cage procedure readily solved the maze problem. Rats trained with the closed metal container procedure did not solve the problem.

These data show environmental information is not sufficient to ensure learning. Rats may need to be oriented in order to construct a single spatial representation of a new environment. Hence, multiple representations or map failure could account for impaired performance. Alternatively rats may encode location by dead reckoning, not distal cues. Dead reckoning could be disrupted by the closed container experience.

761.11

THE INVOLVEMENT OF PASSIVE PATH INTEGRATION IN LEARNING THE MORRIS WATER MAZE. S.H. Alyan,^ D.S. Touretzky^* and J.S. Taube,+ Dept. of Computer Science, Carnegie Mellon University, Pittsburgh, PA 15213^ and Dept. of Psychology, Dartmouth College, Hanover, NH 03755.+

Many previous studies have used the Morris water maze as a tool to investigate the cognitive mechanisms underlying successful navigation (e.g., Morris et al., 1982; Sutherland et al., 1987; Keith & McVety, 1988; Whishaw, 1991). Although some of these studies have questioned the extent to which rats rely solely on the learned relationships between various landmarks in solving the task, none of them have explicitly examined the role path integration plays in learning to locate the platform. Path integration is the ability to compute a trajectory by integrating past passive or active displacements. The present study was designed to determine the effect of degrading passive path integration as the animal learned the water maze.

Four groups of 6 Long-evans male rats underwent training in a 1.9 m diameter water tank containing a partially submerged hidden platform. The rats were transported from their cages to the tank, either on a wooden board in a straight path (Groups 1 & 3) or in a black box in a circuitous path (Groups 2 & 4). Groups 1 & 2 were left on the platform for 30 sec each time before being released into the tank. Groups 3 & 4 were given a total of 17 min placement on the platform before the training began, and no placement on the platform before releasing them into the tank. Latency time to arrive at the platform, percent time spent and number of entries into an annulus zone surrounding the platform were recorded to assess the rats' performance. The rats that underwent a disorientation procedure (Groups 2 & 4) scored significantly lower on all three measures than rats not subjected to disorientation (Groups 1 & 3).

The results suggest that in the water maze, rats navigate by employing a variety of mechanisms simultaneously, including integrating their passive transportation from one location to another. It is not clear how, or how much, each mechanism contributes to successful navigation, but attention should be given to the procedures used in transporting the animal from its home cage to the tank, especially when using this paradigm to test for the functional integrity of underlying spatial mechanisms.

761.12

MORRIS WATER TASK: WHICH CUES DEFINE LOCATIONS? M.P. Weisend*, R.L. Klein, J. M. Hoesing, R.S. Astur, A. Koerner, R.J. McDonald, T. Geving, J. Peinado, J. Biela, J. McWhorter, M. Weems, J. Schlegelmilch, R. Yeo, R.J. Sutherland Dept. of Psychol., Univ. of New Mexico, Albuquerque, NM 87131.

We posed two questions: 1) Do rats swim to locations defined by direction and distance to extrapool cues or pool geometry plus compass direction? and 2) Does the blocking phenomenon occur in place learning? In experiment 1, male and female rats were trained to navigate to a hidden platform at cardinal north about 6 inches from the pool wall. Males received 20 training trials and females 24, two other groups, one of each sex, received 80 training trials. All groups reached asymptotic performance by the end of training. A no-platform probe trial was then conducted. On this probe the pool was rotated 225° with the axis at the hidden platform location (i.e. the pool was moved relative to the room). Male rats with 20 trials searched for the platform in the northern region of the pool, not in the location defined by extrapool cues. In contrast, male rats with 80 trials searched the location defined by extrapool cues, not the north. Female rats with 24 trials searched in the north and the location relative to room cues equally. Females with 80 trials searched in the north. The second study examined the phenomenon of blocking for cues used in place navigation. One group of animals was trained to navigate to a hidden platform with cue set A, then cue sets A and B. The second group was trained with A and B only. On a no-platform probe trial conducted with cue set B only, rats trained with cue sets AB alone performed significantly better than rats trained on A then AB. When further training was conducted with cue set B, only animals trained on A then AB showed a significant increase in escape latency. We conclude that male rats can navigate based on compass direction and pool geometry or direction and distance from extrapool cues depending on training. Female rats seem to use only compass direction and pool geometry under these conditions. In addition, the blocking phenomenon can be demonstrated for cues in a place navigation task.

761.13

LANDMARK-BASED NAVIGATION IN GERBILS SUPPORTS VECTOR VOTING. **L.M. Saksida*, A.D. Redish, C. Reiber Milberg†, S.J. Gaulin†, and D.S. Touretzky.** **School of Computer Science, Carnegie Mellon University, Pittsburgh PA 15213. †Department of Anthropology, University of Pittsburgh, Pittsburgh PA 15261.**

Vector voting and vector averaging are competing hypotheses about how animals use landmarks to reach a goal. A related issue is how cues from multiple reference frames interact when the landmark array moves within a larger, stable environment.

Six male and six female gerbils were trained on a navigation task described by Collett, Cartwright, and Smith (*J. Comp. Physiol*, 1986). A sunflower seed was buried at the center of an equilateral triangle defined by three identical cylindrical landmarks. During training this landmark array was translated after every three trials, but never rotated.

Once the animals reached criterion, no-seed probe trials were intermixed with the training trials. The probe conditions were: (a) normal array, (b) one landmark removed, (c) two landmarks removed, (d) one landmark displaced to twice the normal distance from the goal, and (e) entire array rotated by 180°.

Cumulative 2D spatial histograms of search effort closely resembled those of Collett *et al.* except for the (c) probe, where our animals appeared confused. Performance on the (b), (d), and (e) probes was in agreement with the hypothesis that gerbils predict goal locations by tallying votes from individual landmarks, rather than vector averaging.

We developed two statistical measures to quantitatively analyze the animals' search behavior. Histograms from multiple trials are first aligned based on the landmark array, and searching behavior is allocated to discrete bins. Differences between groups of gerbils or trials are then determined by χ^2 tests. By examining the z-scores of specific bins, we can also demonstrate quantitatively the gerbils' preference for certain locations over others, allowing us to compare the predictions of competing hypotheses.

Probes (b)-(e) produced dramatically more exploration behavior than the (a) case, indicating the gerbils were aware of perturbations to the landmark array. The experiment also revealed that most probe results reported by Collett *et al.* can be obtained without any effort to eliminate stable room cues.

761.14

TWO DIFFERENT MODES OF INTEGRATION OF OLFACTORY AND VISUAL CUES IN SPATIAL ORIENTATION. P. Lavenex (1), P. Gisquet-Verrier*(2), F. Schenk (1). 1-Institute of Physiology, University of Lausanne, CH-1005 Lausanne; 2-NAM CNRS URA 1491, University of Paris Sud, F-91405 Orsay

The aim of this study was to solve the apparent paradox that although macrosmatic, rats orient preferentially relatively to visuospatial cues and ignore olfactory cues when they are in conflict with visuospatial cues. Rats were trained in an eight-arm radial maze with eight different supplementary olfactory cues, while the access to visuospatial cues was prevented. Training was conducted in darkness (infrared illumination), which precluded any visual stimulation, or in translucent tunnels, which restricted the access to visuospatial information but left the inside of the maze illuminated. Rats trained in darkness relied on the olfactory cues and reached maximal performance. In contrast, rats trained in translucent tunnels did not rely on the olfactory cues and their performance remained poor. Nevertheless, probe trials revealed that rats of both groups had memorised the location of the olfactory cues. A third condition, in which olfactory cues were replaced by intramaze visual cues in the translucent maze, revealed that visual but not olfactory cues allowed maximal efficiency in the translucent condition. Performance of rats trained in darkness when the olfactory cues were dissociated from the external spatial frame of reference revealed that the olfactory cues do not need to be linked to spatial information to allow maximal efficiency. When three of the arms contained the same olfactory cue, only the configuration of the olfactory cues allowed to solve the task. In this case, a good performance was observed on condition that these cues were stable relatively to the external spatial frame of reference. This reference could consist of a directional reference maintained by the integration of vestibular information. Together, these results show that olfactory cues are taken into account when they are coherent with spatial information, but do not allow accurate arms' choice when vision is allowed, even if no visuospatial cues are available. Olfactory cues do, however, allow accurate arms' choice in the absence of vision (darkness). This suggests an implicit use of the olfactory cues when vision is available and an explicit use of these cues in darkness.

761.15

SPATIAL ALTERNATION IS NOT FACILITATED BY TRANSECTION OF THE CORPUS CALLOSUM. D.P. Crowne* and S.P.C. Gray. Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, CANADA N2L 3G1

In *The Psychology of Left and Right*, Corballis and Beale proposed that mutual sharing of mirror-image information by the cerebral hemispheres confounds the discrimination of left and right stimuli, so that an animal with intact and communicating hemispheres will learn mirror-image tasks more slowly than one with a divided brain. Discriminating mirrored left and right responses should be similarly affected. Noonan and Axelrod examined the latter, comparing splitbrain and intact rats on a conditional spatial discrimination. Their splitbrain animals were markedly better. Here, we studied another, more basic spatial problem that requires mirrored left and right responses: delayed spatial alternation. Splitbrain (n= 11) and control (n = 11) rats were trained on spatial alternation in a water T-maze similar to Noonan and Axelrod's and tested for 800 trials. The mean trials to criterion of controls was 449.09, of splitbrains 692.73, F (1, 18) = 9.74, p = .006. All controls learned; 7 splitbrains failed to learn. It appears that only some mirror-image spatial tasks are better performed by splitbrain animals, perhaps those involving a conditional cue. The more general effect of callosum section on spatial learning is a severe deficit, earlier shown on allocentric tasks and now on an egocentric one.

761.16

Thiamine Deficiency in 16 month old rats versus 3 month old rats: Behavioral and pathological differences. L.M. Savage*, R. Castillo, & P.J. Langlais. Department of Psychology, SUNY-Binghamton, San Diego State University, and VA Medical Center San Diego, 92161.

To examine age-dependent differences in the neuropathology and behavioral consequences of Pyrithiamine-Induced Thiamine Deficiency (PTD) a group of older rats (16 months) than those used in previous studies (3 months) were pre-trained on the Morris water maze, exposed to either a bout of PTD or Pair-fed (PF) control treatment, re-trained on the water maze, and then tested on an acoustic startle task. A standard bout of PTD produced a higher mortality rate (60%) in these older rats than previously observed in young rats (20%). Furthermore, after the reversal of PTD treatment, the older rats also took twice as long (4 versus 2 weeks) to regain weight lost during treatment, relative to young PTD treated rats. When re-tested on the Morris water maze, the older PTD rats had longer latencies to reach the platform compared to their last pre-training session or relative to the older PF rats on re-acquisition. This "retrograde loss" was not observed in a previous study using 3 month old PTD treated rats. There were no groups difference observed on the acoustic startle task. The neuropathology produced by PTD treatment in the older rats is different from what has been seen in young PTD-treated rats. Thus, the age at which a rat is exposed to PTD treatment appears to influence both the behavioral and pathological consequences.

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761.17

EFFECTS OF NEONATAL OXYGEN DEPRIVATION ON DEVELOPMENT OF SPATIAL LEARNING IN RATS. R.E. Hartman* and C.R. Almli. Developmental Neuropsychobiology Lab., Dept. Psych., Washington University, St. Louis, MO 63108.

Human neonatal oxygen deprivation has been related to a wide range of developmental disabilities, including cerebral palsy, mental retardation and behavioral disorders. The present study determines the effects of neonatal oxygen deprivation on the development of spatial learning and memory in the albino rat. At postnatal ages (days) PN 0 to PN 7, pups were subjected to 3 consecutive periods of oxygen deprivation (gasping criteria) separated by 30-min in room air.

Beginning on PN 18, control and oxygen-deprived (OD) rats were tested in a Morris water maze for 10 consecutive days. Three conditions were tested: distal/spatial (invisible escape platform in a fixed location), proximal/cue (visible platform in a fixed location), and random (invisible platform placed in random locations). Each of the 10 testing days consisted of 12, 60-sec acquisition trials (the maze contained an escape platform), and a 60-sec retention trial (the maze did not contain an escape platform). For acquisition trials, latency to enter the maze quadrant containing the platform, and latency to find the platform, were recorded (maximum score = 60-sec). For the retention trials, latency to enter the quadrant that previously contained the platform, and total amount of time spent in that quadrant, were recorded (maximum score = 60-sec).

During acquisition trials, OD rats escaped the maze faster than control rats under the distal/spatial condition. During retention trials, however, control rats in the distal/spatial condition spent more time in the quadrant that previously contained the platform than did OD rats. It appears that neonatal oxygen deprivation may differentially affect acquisition and retention of spatial abilities. Acquisition escape latency may have been faster for OD rats via hyperactivity, whereas retention for the platform's location was adversely affected. These results may be related to the development of glutamate/NMDA receptor excitotoxity.

761.18

ALTERED ATTENTION, DECLARATIVE MEMORY, AND REWARD EFFICACY IN RATS EXPOSED TO LEAD DURING EARLY DEVELOPMENT: EVIDENCE FOR DYSFUNCTION OF PREFRONTAL CORTEX, HIPPOCAMPUS, AND NUCLEUS ACCUMBENS. B.J. Strupp*, T. Braga Costa, S. Drazen, C. Hu, H. Raffman, S. Alber, L.E. Bayer, & D. Levitsky. Dept. of Psychology & Div. of Nutritional Sciences, Cornell Univ., Ithaca, NY 14853.

Despite the increasing evidence that low-level lead (Pb) exposure lowers IQ, relatively little is known about the specificity of the impairment. This presen-tation will focus on 3 tasks included in an investigation designed to provide insight into this issue. Pb exposed S's displayed impaired inhibition of prepotent res-ponses in a vigilance task, consistent with prefrontal cortex (PFC) dysfunction. In a win-shift radial maze task, both Pb-exposed groups were significantly impaired relative to controls when tested after an 8-hr retention interval; at shorter delays, they were either unimpaired or superior to controls. This pattern demonstrates a specific impairment in declarative memory, consistent with the evidence that Pb exposure alters hippocampal LTP and NMDA receptor function. This latter task and a matching-to-position task provided evidence that developmental Pb exposure produces a lasting increase in the rewarding value of reinforcers. The increased incentive and impaired response inhibition may both be related to altered dopamine (DA) activity, in nucleus accumbens and in PFC respectively. The evidence that DA activity in PFC inhibits accumbens DA activity suggests that the observed alterations in executive function and incentive motivation may be linked. It is notable that changes in these two functional domains are also seen following prenatal alcohol exposure and in ADHD.

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761.19

PLACE RETENTION, EXPLORATION, HABITUATION, AND SEROTONIN IN THE RAT. G. Wörtwein, P. Plenge, E. Mellerup and J. Mogensen*. Lab. of Neuropsychiatry, Dept. of Pharmacology, University of Copenhagen and Rigshospitalet-6102, The University Hospital, Copenhagen, Denmark.

A selective lesion of the serotonergic system was made by intracerebroventricular injections of 0.1mg 5,7-dihydroxytryptamine in 10μl 0.1% ascorbic acid per side. Selectivity was assured by pretreatment with normifensine and desimipramine. Postoperatively, habituation of locomotion in an activity cage, habituation of exploration in a vertical holeboard apparatus and retention of a place-learning task in a water-maze were tested.

Quantitative receptorassay of the serotonin reuptake site confirmed the lesion by showing a B_{max} reduction of more than 90%. While habituation of locomation was not affected by this almost complete elimination of the serotonergic system, habituation of exploration was impaired. Furthermore we found place recall to be significantly impaired. Finally, a pharmacological challenge by scopolamine (0.5mg/kg) demonstrated that the place recall of the lesioned animals was hypersensitive to muscarinergic receptor blockade.

These results emphasize the importance of serotonergic and cholinergic interactions for spatial memory - an interaction which may be of importance in Alzheimer's disease.

761.20

EFFECTS OF CHRONIC ANTIDEPRESSANT TREATMENT ON PASSIVE AVOIDANCE BEHAVIOR IN RATS. L.C.Daws*, R.Lopez and A.Frazer. Dept. Pharmacology, Univ. Texas Health Science Center at San Antonio, TX, 78284-7764.

Performance in a passive avoidance (PA) task can be altered by drugs that block ß-adrenergic (BAR) and muscarinic cholinergic receptor systems. The amnesic effect of the muscarinic antagonist, scopolamine, in a PA task can be potentiated by blockade of BARs; blockade of amygdaloid BARs can itself cause an amnesic effect. Chronic antidepressant (AD) treatment of rats results in down-regulation of BARs and this effect occurs robustly in the amygdaloid nuclei, a brain region strongly linked to the regulation of emotionality, particularly fear. Consequently, we determined whether chronic AD treatment in combination with an acute injection of the muscarinic receptor antagonist, scopolamine, would disrupt retention on a PA task. Initial experiments corroborated earlier reports in that: (1) propranolol (34 or 68 nmoles), injected directly into the amygdala complex of rats trained on a PA task, produced dose dependent decreases in retention of the task, and (2) blocking BARs, by systemic administration of propranolol (10 mg/kg, i.p.), potentiated the amnesic effect of scopolamine in a PA task.

Rats were treated with either phenelzine (5 mg/kg, i.p.) daily for 21 days, or desipramine, (10 mg/kg, i.p., bid) for 8 days. Control animals received vehicle at corresponding times. Twenty minutes prior to acquisition training [electric footshock, 0.5 mA for 2 secs (phenelzine group), or for 3 secs (desipramine group)] each of the three treatment groups were randomly divided and given an acute injection of either scopolamine (0.15 or 0.3 mg/kg, i.p.) or saline. Retention (10 min. test) was recorded 24 hours later. All groups showed maximal or near maximal retention on the test day. There was a tendency for rats receiving chronic AD treatment prior to scopolamine to exhibit decreased retention latencies, but these differences were not significant. Chronic treatment of rats with ADs does not alter performance or potentiate the effect of scopolamine on a PA task. Unlike blocking amygdaloid BARs with propranolol, AD-induced down-regulation of such receptors does not produce an amnesic effect in a PA task. (Supported in part by Research Funds from the VA and USPHS Grant MH 29094).

762.1

CHALLENGING IBOTENIC ACID LESIONING:
A NEUROANATOMICAL TOOL TO ASSESS THE INTEGRITY OF FIBERS OF PASSAGE IN BEHAVIORAL-LESION STUDIES.
S. Frey*, R. Morris and M. Petrides. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada.
In behavioral-lesion studies involving animals, ibotenic acid (IBO) is widely used as a means of damaging brain structures. IBO is considered to be the lesioning method of choice since it destroys cell bodies but leaves fibers of passage intact. If IBO, however, is not used properly, i.e., if the concentration or the volume are too high when injected in a given area, both cell bodies and fibers of passage will be destroyed. The present project evaluates, first, whether ibotenic acid (IBO) is a reliable means of destroying a specific neural structure, and, second, whether it really leaves fibers of passage intact through the lesioned area. In 20 rats, the central nucleus of the amygdala was destroyed with IBO and the fibers that pass through this brain region were evaluated by means of an anatomical tract-tracing technique, namely HRP-WGA histochemistry. The results of this study have shown that IBO could be an ideal means of destroying cell bodies in a given brain area while at the same time sparing fibers running through that area. However, anatomical tract-tracing methods should be considered to assess the success of this technique. In light of the present results, behavioral-lesion studies would benefit from applying this reliable yet simple technique.

762.2

IMMUNOTOXIN-INDUCED CHOLINERGIC LESION IN INFANT RATS. B.A. Pappas*, C. Davidson, S. Nallathamby, G.A.S. Park, R.G. Wiley and T. Fortin. Life Sciences Research Centre, Carleton Univ., Ottawa, Ont. K1S 5B6.

Seven day old rat pups received bilateral intraventricular injections of the cholinergic immunotoxin IgG-192 saporin (200 ng/1.5 µl/ventricle). Four to six months later the immunotoxin-injected rats showed profound (88%) reduction of choline acetyltransferase activity in the hippocampus and lesser reductions in cortex (51%) and caudate (30%). Forebrain monoamines were unaffected except for increased NE in cortex and DA turnover in hippocampus. Immunohistochemical labelling of the p75 NGF receptor a marker for basal forebrain cholinergic neurons, indicated reduced immunoreactive neurons which varied among animals. Grossly enlarged immunoreactive varicosities were observed in the dentate gyrus and to a lesser extent in CA2 and CA3. Despite profound reduction and abnormality of hippocampal cholinergic innervation, the IgG treated rats showed neither a spatial memory impairment on the Morris water task, or a short term memory impairment on a delayed spatial alternation task. (Supported by NSERC).

762.3

VISUO-SPATIAL LEARNING IN MICE EXPOSED TO PRENATAL GAMMA RADIATION. R.W.F. Vital* (1), C. Moreira (1), M.L.Dutra (1), R. Linden (2) and S.L. Schmidt (1,3). (1) Departamento de Ciências Fisiológicas, Universidade do Estado do Rio de Janeiro, RJ, 20551030, Brasil. (2) Instituto de Biofísica, Universidade Federal do Rio de Janeiro, Brasil. (3) Department of Psychology, University of Alberta, Edmonton, Canada.

Exposure of pregnant mice to gamma radiation at 16 days of gestation (E16) produces in the progeny extensive shrinkage of the occipital cortex and disruption of the laminar pattern of the hippocampus. This investigation was designed to study visuo-spatial learning in irradiated animals. Pregnant females were exposed to a gamma source at E16 with a total dose of 3Gy. Spatial learning of 58 adult mice (n = 28 irradiated progeny and n = 30 non-irradiated animals) was assessed in the Morris water maze task. This behavioral testing was conducted on two consecutive days, with each mouse receiving 5 trials per day. After 1 week, 20 animals (n=10 irradiated progeny, n=10 non-irradiated) were also tested in the Lashley III Maze. In this test each animal received ten trials, one trial per day, Analysis of the Morris water maze showed that there was not a significant difference between irradiated and non-irradiated animals on the first trial of the first day. On the subsequent trials, the time to reach the hidden platform was consistently greater in the irradiated animals as compared to the normal mice. Similarly, analysis of the Lashley III Maze showed a significant difference between the two groups after the fourth trial. After this trial, normal mice learned the task whereas the irradiated animals never reached the learning criterion. These data show a deficit in the visuo-spatial learning of the irradiated animals and may be a consequence of the early lesion in the hippocampus.

762.4

NEONATAL VENTRAL HIPPOCAMPAL LESIONS IMPAIR SPATIAL LEARNING AND MEMORY IN THE RADIAL-ARM MAZE AND ATTENUATE RESPONSE TO THE NICOTINIC ANTAGONIST MECAMYLAMINE. R.A. Chambers, J.P. McEvoy and E.D. Levin*. Neurobehavioral Research Laboratory, Psychiatry Department, Duke University Medical Ctr., Durham, NC 27710 USA.

In a rat model of schizophrenia developed by Lipska et al. (Neuropsychopharmacology 9:67-75, 1993), ibotenic acid lesions are made in the ventral hippocampus on Day 7 after birth, which results in locomotor hyperactivity with an onset during adolescence. We studied the cognitive effects of this lesion by examining spatial learning and working memory in the radial 8-arm maze. Training for 18 sessions began on Day 40 with 6 lesioned and 14 sham operated male and female Sprague-Dawley rats. The lesioned rats had significantly ($p<0.025$) lower accuracy (entries to repeat, Control=6.4±0.1 and Lesioned=5.8±0.2, mean±SEM). The lesion effect became evident with continued training (lesion x session, $p<0.025$). No significant lesion effect was seen on latency. After acquisition, challenges with nicotine (0.1-0.4 mg/kg), mecamylamine (2.5-10 mg/kg) and scopolamine (0.4-1.6 mg/kg) were conducted. Lesioned rats showed a significantly attenuated amnestic response to the nicotinic antagonist mecamylamine ($p<0.025$), suggesting that this system was less critical in lesioned rats than controls for performance of the memory task. These results demonstrate that in addition to hyperactivity, ventral hippocampal lesions on Day 7 cause long-term deficits in spatial learning and working memory function. This may provide a model for the cognitive deficits seen in schizophrenia. (This research was supported by the National Alliance for Research on Schizophrenia and Depression.)

762.5

ACUTE INJECTIONS OF QUINOLINIC ACID CAUSE DOSE -DEPENDENT SPATIAL MEMORY DEFICITS IN RATS.
G.L. Dunbar*, M.C. North, K.L. Haik, and J. Dong. Brain Research Laboratory, Department of Psychology, Central Michigan University, Mt. Pleasant, MI 48859.

Previous work has indicated that acute bilateral injections of quinolinic acid (QA) can mimic some of the neuropathological conditions observed in Huntington's disease and can produce profound learning deficits in a Morris water maze task. However, it was not previously known whether acute bilateral injections of QA could cause memory deficits in a well-learned spatial memory task. The present study investigated this possibility. Six groups of rats were given acute bilateral intrastriatal injections of QA at 0, 100, 125, 150, 175, and 200 nmol concentrations following acquisition of a radial-arm water maze spatial learning task. Although rats with the higher doses of QA took significantly longer to reacquire the task, all rats eventually were able to relearn the task. These results indicate that while acute bilateral intrastriatal injections of QA can disrupt spatial memory in rats, the memory deficits produced are not permanent and are not as profound as those observed in acquisition tasks.

762.6

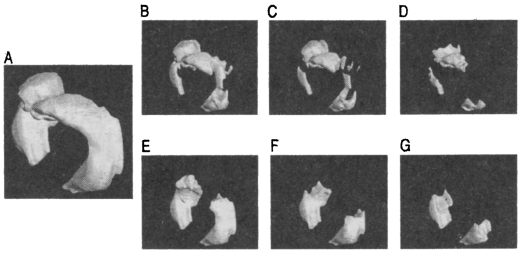
THE HIPPOCAMPUS CONTRIBUTES TO SPATIAL LEARNING AND MEMORY IN RATS WITH FIMBRIA/FORNIX LESIONS. D. K. Hannesson* & R. W. Skelton. Dept. of Psychol., Univ. of Victoria, B.C., Canada, V8W 3P5.

Fimbria/fornix (FF) lesions consistently produce impairments in a variety of spatial paradigms but do not prevent spatial learning entirely since FF-lesioned animals can utilize spatial solutions in such tasks when given extensive training. Though the anatomical substrates of this preserved capacity are unknown, a plausible hypothesis is that the hippocampus continues to subserve some of its normal function since both its internal circuitry and cortical connections remain largely intact after FF lesions. The present study investigated this hypothesis by examining the role of the hippocampus major cortical input/output pathway, the perforant path (PP), in spatial learning and memory after FF lesions in rats (*Rattus rattus*). In Experiment 1, rats subjected to FF lesions (FF), combined FF and PP lesions (FFPP), or sham operations (SH) were tested for acquisition of a constant platform location in the Morris water maze. In agreement with previous findings, FF lesions produced a severe impairment in the rate of acquisition of the platform's location but did not prevent it. Concurrent PP lesions aggravated this impairment but still permitted some spatial learning. In Experiment 2, these same rats received either a PP-lesion or sham-operation (creating groups FF/PP, SH/PP, FF/SH, SH/SH, and FFPP/SH), and were then tested for retention of the platform's location. PP lesions impaired retention in FF-lesioned rats and produced a more modest deficit in sham-operated rats. However, both groups' performance improved to the levels of their respective controls (FF/SH, SH/SH) with further training. No serial lesion effect was observed as both FF/PP and FFPP/SH performed at comparable levels. Our results suggest that the hippocampus does contribute to residual spatial learning and memory abilities after FF lesions. However, since ventral portions of the PP were spared by our lesion procedure, it cannot be determined whether spared hippocampal connections or alternative structures were responsible for residual function following combined FF-PP lesions.

762.7

SPATIAL LEARNING WITH A MINISLAB IN THE DORSAL HIPPOCAMPUS. M.-B. Moser*, E. I. Moser, E. Forrest, P. Andersen and R.G.M. Morris, Centre for Neuroscience, Univ. Edinburgh, Edinburgh EH8 9LE, U.K., and Dept. of Neurophysiology, Univ. Oslo, Norway.

The volume and location of hippocampal (HPC) tissue required for normal spatial learning in rats was investigated. Ibotenic acid was used to make bilateral, symmetric lesions of 20 -100% of HPC volume (Fig1 B-G), with the spared tissue as blocks at either the dorsal or ventral pole of HPC. Spatial watermaze learning was more efficient when the residual tissue was in the dorsal than in the ventral HPC. Even small transverse slabs of HPC [>26 % of total; 1D) could support spatial learning provided they were in the dorsal pole, whereas 60-80 % had to be spared to achieve normal spatial learning with blocks starting from the ventral pole (1E). Acetylcholinesterase staining, population spikes and synaptic plasticity in the remaining dorsal and ventral slabs were inside normal ranges. Thus, HPC-dependent spatial learning only requires the integrity of only a minislab of dorsal HPC tissue.



762.8

PARTIAL HIPPOCAMPAL KINDLING CAN PRODUCE A TEMPORARY DEFICIT IN PLACE LEARNING BY RATS IN THE MORRIS WATER TASK. R. J. Sutherland*, L. S. Leung, R. J. McDonald, & M. P. Weisend. Depts. of Psychol., Univ. of New Mexico, Albuquerque, NM, and Depts. of Physiol. & Clin. Neurol. Sci., Univ. of Western Ontario, London, Canada.

Partial hippocampal (HPC) kindling has been shown to affect performance by rats in the radial arm maze task for about 3 weeks after stimulation (Leung, et al. Behav. Brain Res., 40: 119-129, 1990). We sought to extend these observations in another task, the Morris water task, which is also known to be sensitive to damage in the hippocampal formation. We conducted two experiments to determine if induction of 20 HPC afterdischarges (ADs) affects place navigation in rats.

First, we implanted 12 rats with electrodes bilaterally in the CA1 region. Half of the rats received 21 ADs (1 sec train of 100 µs duration pulses at 100 Hz); the others received only low frequency stimulation. They were trained in the fixed location, hidden platform version of the Morris water task, receiving 8 trials on days 1, 2, 3, 7, 14, and 21 after the last stimulation. Partially-kindled and control rats learned at comparable rates and attained a similar level of performance. Second, we trained 18 rats in the hidden platform, moving location version of the task before delivering stimulation. After the last session we tested their performance in the original pool room and a new room. We found a small, but statistically significant, deficit in place learning set in the old and new rooms, 1 day and 1 wk, but not 3 wks after partial kindling.

These results extend the conclusion that in tasks sensitive to hippocampal damage, including the radial arm maze and place learning in the Morris water task, partial hippocampal kindling can disrupt performance for many days. (Supported by NIH 25383)

762.9

IMPORTANCE OF THE HIPPOCAMPUS DENTATE-HILAR COMPLEX FOR LEARNING AND MEMORY IN THE RAT. W. Samuel#, E. Brush, E. Masliah#, M. Garcia-Munoz, P. Patino, S. Young, P. Groves*. Depts. of Neurosciences# and Psychiatry, Univ. of Calif. Sch. of Med., San Diego, CA 92093-0624.

Prior studies of postmortem tissue from the hippocampal formation (HF) found lesions in the dentate-hilar area to be particularly correlated with Alzheimer's dementia, and prior animal work has suggested that lesions to this area might be particularly disruptive of learning and memory in the rat. As a test of this hypothesis, 30 male rats 50 days of age were trained to press a lever to obtain sucrose pellets on an FR3 schedule only when a stimulus light was off. At 80-82 days of age 10 animals received bilateral injections of 0.2 ul ibotenic acid (20 mM) in the anterior and posterior dentate gyrus/hilar region (DG/H), 10 other animals matched for learning ability were injected with ibotenic acid in the anterior and posterior cornu ammonis (CA2/3), and 10 underwent sham lesions. Eight days after surgery, animals were tested for 30 minutes with the FR3 light discrimination task. On all performance measures, the DG/H group displayed the greatest impairment and the sham controls the least, with the CA2/3 group falling in between: minutes to attain an FR3 criterion performance ($p< .006$), minutes maintaining an FR3 criterion ($p<.04$), ratio of light on to light off bar presses ($p<.004$), and number of reinforcements ($p<.06$). Five to 8 weeks later, rats underwent a one hour trial on an alternating response task involving two response levers, which required the animal to press the left bar (the one previously present) and then the right bar in order to obtain a pellet. The rats exhibited a high rate of bar pressing, but it was nonetheless very difficult for them to learn the alternating sequence. The DG/H group had the most impairment, both in terms of obtaining reinforcements ($p<.005$) and in terms of the ratio of left bar presses to reinforcements, a measure of inappropriate perseveration ($p<.03$). Following perfusion and sacrifice, histology with H&E and immunocytochemistry with glial fibrillary acidic protein confirmed the accuracy of the bilateral lesion placements.

762.10

FIMBRIA/FORNIX LESIONS ENHANCE PERFORMANCE ON A NON-SPATIAL TRAJECTORY TASK IN THE WATER MAZE. A.M. White, D.B. Matthews, P.J. Best*. Dept. of Psychology and Center for Neuroscience, Miami University, Oxford, OH 45056

Lesions of hippocampal connections, such as the fimbria-fornix, impair performance on tasks that require the formation of a spatial-cognitive map, but spare the ability to solve non-spatial tasks. A number of studies on the radial-arm maze have found that rats with fimbria-fornix lesions actually perform better than intact rats on various non-spatial tasks. However such has never been reported in the Morris water maze, perhaps because the non-spatial tasks previously used have been too easy.

The current study compares performance of intact and fimbria-fornix lesioned rats on a spatial task and a relatively difficult non-spatial trajectory task in the Morris water maze. Subjects were started from four different locations on each of seven days of training. In the spatial task, the platform location remained fixed with respect to the training room, regardless of the start location. In the trajectory task, the platform location remained fixed with respect to the start location (i.e. to the left of the start position), regardless of the position in the room. The results show that lesioned animals were impaired on the spatial task ($p<.05$) but performed significantly better than controls on the trajectory task ($p<.05$). The results suggests that the formation of a spatial cognitive map by the intact animals placed them at a disadvantage on the non-spatial trajectory task. The fimbria-fornix lesioned rats could not form such a map, and therefore did not suffer the same disadvantage on the trajectory task.

762.11

SELECTIVE IMPAIRMENTS OF SPATIALLY-MEDIATED BEHAVIORS PRODUCED BY TRANSIENT, LIDOCAINE-INDUCED LESIONS OF THE VENTRAL HIPPOCAMPUS OF THE RAT. A.G. Phillips, S.B. Floresco, J.K. Seamans and R.C. Tees* Dept.of Psychology, University of British Columbia, Vancouver, B.C., V6T 1Z4

The ventral hippocampus (VH) is part of a neural circuit which includes the prelimbic region (PL) of the medial prefrontal cortex and the nucleus accumbens (N.Acc.). Previous reseach has shown that the N.Acc. (Seamans and Phillips, *Behav.Neurosci.*, 1994(108) 456-468) but not the PL (Seamans, Floresco and Phillips, 1994, *Society for Neuroscience abstracts*, 494.4) is essential for spatially-mediated foraging behavior in the rat. The purpose of the present study was to determine the unique contribution of the VH to spatially-mediated behaviors. Well trained rats with lidocaine-induced lesions of the VH made more revisit errors (relative to saline controls) on a radial arm maze task, in which 4 out of 8 arms were baited randomly. Lesions in a separate group of rats produced impairments in the acquisition of a water maze task, but did not effect retention of spatial information on this task at a time when the anesthetic effects of lidocaine had dissipated. Transient lesions of the VH prior to retention of a water maze task produced no impairments in performance. Taken together, these results suggest that 1) the VH is not involved in the retention of spatial information of a water maze task, but 2) the VH is involved in "on-line" processing of spatial information during foraging on a radial arm maze or acquisition of a water maze task. Given the result of our previous studies, it is proposed that the VH specifically interacts with the N.Acc, but not PL, to guide spatially cued foraging behavior.

762.12

SELECTIVE IMPAIRMENT ON A DELAYED RADIAL ARM TASK FOLLOWING LOCAL ADMINISTRATION OF A D1 BUT NOT D2 ANTAGONIST INTO THE PREFRONTAL CORTEX. J.K. Seamans*, G.R. Wunderlich, S.B. Floresco & A.G. Phillips Dept.of Psychology, University of British Columbia, Vancouver, B.C., V6T 1Z4

Recently we have demonstrated a preferential involvement of the prelimbic region (PL) of the rat medial prefrontal cortex in delayed but not single phase foraging behavior. Using an in vitro slice preparation we also have shown that neuronal activity in this region is modulated by dopamine, primarily via stimulation of the D1 receptor (Yang & Seamans, *Society for Neuroscience abstracts*, 1995). In order to gain insight into the behavioral consequences of dopaminergic modulation in the PL, D1 or D2 antagonists were delivered locally into this region prior to the performance of a delayed or single phase foraging task on an 8-arm spatially-cued radial maze. The D1 antagonist SCH-23390 (0.05, 0.5, 5ug/ 0.5ul), but not the D2 antagonist sulpiride (0.05, 0.5, 5ug/ 0.5ul), dose-dependently disrupted performance on the delayed spatial win-shift task in which the rats were required to visit 4 open and baited arms during a Training phase and to visit the 4 remaining arms that were baited 30min later during a Test phase. Rats receiving SCH-23390 injections made an equal number of across phase errors(revisits to arms visited during the Training phase) and within-phase errors(revisits to arms visted within the Test phase), indicating disorganization of the effecient foraging strategy. In contrast, similar doses of these drugs had no effect on performance of the random foraging task in which 4 of 8 arms were baited randomly each day. These results compliment data obatined in the primate, and demonstrate that the D1 but not the D2 receptor in the PFC is essential for accurate performance on tasks in which information acquired prior to a delay is used to organize prospective responses.

762.13

THE ROLE OF THE HIPPOCAMPUS IN REDUCING SPATIAL INTERFERENCE. W.E. DeCoteau*, R.P. Kesner and P. Gilbert. Dept. of Psychology, University of Utah, Salt Lake City, UT 84112

A delayed-match-to-sample for spatial location task on the dryland version of the Morris water maze was utilized to assess spatial interference in rats. During the task's study phase an object which covered a baited food well was randomly positioned in one of six possible spatial locations. Rats exited a start box and displaced the object in order to receive the food reward and were then returned to the start box. On the ensuing test phase rats were allowed to choose between two objects which were identical to the study phase object. One object was baited and positioned in the previous study phase location (correct choice), the other was unbaited and placed in a different spatial location (incorrect choice). Five distances (min = 15 cm, max = 105 cm) were randomly used to separate the foil from the correct object. Following the establishment of a criterion of 75% correct averaged across all separation distances, rats were given either hippocampal or cortical control lesions. Post-surgery control animals matched their pre-surgery performance for all spatial distances. Hippocampal lesioned animals displayed impairments for short (15 cm - 37.5 cm) and medium (60 cm) spatial separations, but performed as well as controls when the spatial separation was long (82.5 cm - 105 cm). These results suggest that the hippocampus plays a significant role in reducing the interference produced by proximally close spatial locations.

762.14

HIPPOCAMPAL LESIONS AND TEMPORAL PROCESSING IN THE PIGEON: THE USE A DURATION MATCHING-TO-SAMPLE PROCEDURE TO EXAMINE HIPPOCAMPAL FUNCTION. J.M. Dose, P.J. Kraemer, C.K. Randall, R.W. Brown & J.F. Zolman*. Depts. of Psychology and Physiology, University of Kentucky, Lexington, KY 40506.

The role of the avian hippocampus in subjective timing and short-term memory of temporal events was studied using a duration matching-to-sample (MTS) procedure. Pigeons were initially trained on two duration MTS problems in succession. Samples consisted of 2- and 10-s durations of a red light presented from the front wall or white light presented from the ceiling. Choice stimuli consisted of red, blue, green and yellow solid colors presented on two side keys. Correct choices were followed by 2-s access to grain before a 15-s ITI commenced; incorrect choices immediately produced the 15-s ITI. Before and after receiving bilateral aspiration lesions of the hippocampus, pigeons were tested with normal (nonprobe) and probe trials in both the subjective timing and short-term memory tasks. Nonprobe trials for both test sessions consisted of red and white samples immediately followed by appropriate choice stimuli. Test sessions of subjective timing included a random presentation of 40 nonprobe and 8 probe trials. These probe trials consisted of red or white samples presented for 3-, 5-, 7-, or 9-s. Compared with pre-lesion performance, pigeons were more likely to respond "long" following hippocampus lesions, but this effect differed as a function of sample color. Test sessions of short-term memory included a random presentation of 24 nonprobe and 24 probe trials. These probe trials also involved 2- or 10-s presentations of red or white light; but, 0-, 1-, 3-, 6-, 9-, or 12-s delays were imposed between signal offset and choice stimuli onset. Pre- and post-lesion accuracy was disrupted on long samples following the retention interval but this effect was not found using short samples. Results will be discussed in terms of changes in temporal processing following a disruption of normal hippocampus functioning.

762.15

THE EFFECTS OF HIPPOCAMPAL LESIONS IN PIGEONS: VISUAL DMS WITH INTERFERENCE AND SPATIAL MEMORY. M. Colombo* and S. Cawley. Department of Psychology, University of Otago, Dunedin, New Zealand.

Previously (SN Abstracts, 20, 1012) we reported that hippocampus and area parahippocampalis (Hp-APH) lesions in pigeons had no effect on the postoperative retention or acquisition of a visual DMS task, or the postoperative acquisition of a concurrent discrimination task. Given that Hp-APH lesions cause deficits in homing behavior, these results suggested that the hippocampus in pigeons functions mainly to support the processing and retention of spatial, rather than visual, information.

A number of primate studies have reported that the effects of hippocampal lesions are exacerbated under conditions of interference. In this current study we explored whether visual DMS behavior in pigeons was impaired by testing with two forms of interference. The same ten subjects that served in the previous report served in the current studies. Five had received bilateral Hp-APH lesions and five served as unoperated controls. In Experiment 1, we examined the effects of proactive interference on visual DMS behavior by testing the pigeons with either a 15 sec or 1 sec intertrial interval (ITI). Confirming previous studies, reducing the ITI led to an impairment in performance. However, both control and Hp-APH pigeons were equally affected. In Experiment 2, we examined the effects of retroactive interference by interjecting visual interference during the delay period. Visual interference reliable impaired performance in both control and Hp-APH pigeons. Again, however, both control and Hp-APH pigeons were equally affected. In Experiment 3, we examined the effects of Hp-APH lesions on the acquisition of a open-field radial maze task. Of the five birds tested to date, three Hp-APH pigeons required 38, 41, and 47 days to learn the task, whereas two control pigeons learned the task in 6 and 14 days. These results provide further evidence that the pigeon hippocampus is important for the processing and retention of spatial rather than visual information.

762.16

SEASONAL DIFFERENCES IN FOOD-STORING, BUT NOT SPATIAL MEMORY OR HIPPOCAMPAL SIZE IN WHITE-BREASTED NUTHATCHES. K. Petersen and D.F. Sherry*. Dept. of Psychology, Univ. of Western Ontario, London, Ontario, Canada, N6A 5C2.

Several recent studies have described seasonal differences in hippocampal size and neurogenesis that are correlated with seasonal changes in food-storing behaviour. In Black-capped chickadees, both the relative size of the hippocampus and the number of [3H] thymidine labelled neurons in the hippocampus were found to be greatest in October, which coincides with the onset of food-storing (Barnea & Nottebohm, *PNAS* 1994 91, 11217; Smulders et al. *J. Neurobiol.* 1995 27, 15). We wished to determine whether seasonal differences in the size of the hippocampus coincided with seasonal changes in food-storing and spatial memory in the food-storing White-breasted nuthatch (*Sitta carolinensis).*

Birds were captured in the wild between October and April. Food-storing behaviour was observed in an outdoor aviary for two days. Accuracy of memory for caches was determined during five days of observation in an indoor aviary. Birds were sacrificed and volume of the hippocampus, telencephalon, and lobus parolfactorius were determined. Behavioural results indicated that birds in the winter months had significantly higher levels of caching and cache retrieval than spring birds. No seasonal differences were found in the accuracy of memory for cache sites. Neuroanatomical results indicated a larger telencephalon in spring than in winter, but no seasonal difference in relative size of the hippocampus, or lobus parolfactorius. Thus, no seasonal difference in spatial memory or relative size of the hippocampus was found to coincide with the observed seasonal change in food-storing activity.

762.17

PLACE LEARNING ON THE MORRIS WATER TASK DOES NOT REFLECT CA1 SYNAPTIC DENSITY CHANGES ACROSS THE ESTROUS CYCLE. S.G. Warren* and J.M. Juraska. Dept. of Psychology, University of Illinois, Champaign, IL 61820.

Synaptic density and long term potentiation (LTP) in hippocampal CA1 stratum radiatum vary significantly across the rat estrous cycle. The current study examined the behavioral implications of these rapid neural changes across the estrous cycle. While previous studies have suggested that spatial learning varies between the behavioral estrous and diestrus phases of the cycle no attempts were made to minimize the stressful aspects of the task, even though the stress response varies significantly across the estrous cycle. In the current study females in each phase of the estrous cycle and males were trained on a place version of the Morris water task, with each female's training restricted to one phase of the cycle. To reduce the stress involved with performance on this task all animals received extensive handling and pre-training (in a different room) in a small water maze with a visible platform. Results indicated that performance varied across the estrous cycle. On several measures females in estrus were similar to males, while rats in diestrus were similar to those in proestrus, with females in proestrus performing more poorly than males and females in estrus. The anatomical and LTP changes across the cycle do not correlate with the behavior. Supported by NSFIBN9310945 to JMJ and HD07333 to SGW.

762.18

THE ESTRUS CYCLE AFFECTS SPATIAL LEARNING IN ANIMALS WITH HIPPOCAMPAL DAMAGE. B. Therrien*, E. K. Hebda-Bauer, N. J. Bidlack, The University of Michigan, Ann Arbor, MI 48109-0482

Damage to the hippocampus (HPC) produces greater place navigation deficits in females than males. We also find that female animals with HPC damage are more resistant to therapy and more distractible than males. This study examined the influence of the estrus cycle on spatial learning in the presence and absence of HPC damage. We hypothesized that the stage of estrus on the initial test day influences spatial learning on subsequent days as prior work has shown that the majority of learning occurs between the first and second day of behavioral testing. Adult female (N = 32) rats received bilateral HPC lesions or sham surgery (control). Upon recovery, all animals were given 4 days of testing on the Morris water task. Daily vaginal smears determined the stage of estrus cycle. As expected, behavioral testing revealed that animals with bilateral damage were markedly impaired when compared to controls ($p<.05$). Stage of the estrus cycle did not significantly affect performance in controls. In contrast, lesioned animals in proestrus (proestrus and early estrus: high estrogen levels) learned the place navigation task more efficiently than those in diestrus (metestrus and diestrus: low estrogen levels), as evidenced by shorter swim times on the first 2 days (day 1: $X = 71.06 \pm 13.98$ vs $X = 114.09 \pm 19.96$, $p = .10$ and day 2: $X = 39.25 \pm 14.01$ vs $X = 91.03 \pm 14.61$, $p<.05$). The groups continued to differ across all 4 test days. The extent of HPC damage did not differ between the two lesioned groups. Qualitative analyses of swim paths revealed that lesioned animals in proestrus displayed more purposeful psychomotor learning strategies compared to the perseverative patterns of females in diestrus. These results show that animals with HPC damage quickly adopt more efficient learning strategies when estrogen levels are high. We therefore conclude that spatial learning is enhanced by peak estrogen levels.

762.19

EXPLORATORY BEHAVIOR IMPROVES SPATIAL LEARNING IN FEMALES WITH UNILATERAL HIPPOCAMPAL LESIONS. T. Briones*, C. Pacini, B. Therrien. The University of Michigan, Ann Arbor, MI 48109-0482

Exploratory behavior is thought to enhance spatial learning; such behaviors are reportedly absent in rats following bilateral hippocampal (HPC) damage. Our prior work revealed gender differences in animals with HPC damage. Specifically, females, unlike males, engage in exploration nearly as much as controls when exposed to a novel environment. Although females with bilateral HPC lesions engage in exploration, they still have spatial learning deficit. The purpose of this study was to determine if the association between exploration and spatial learning is a function of the amount of hippocampal damage. Adult female rats were randomly assigned to either unilateral (n = 29) HPC lesion or sham surgery (n = 12). Following recovery, rats were tested for four days on the Morris water task. Exploratory activities, i.e., rearing and circling were recorded for 30 seconds once the rats reached the platform. Rats with unilateral HPC damage engaged in as much or more exploration as controls. When lesioned animals were categorized as having greater than average exploration ($\bar{X}$ activity >12 epochs) vs average exploration ($\bar{X}$ activity <12 epochs) on test Days 1 and 2, a significant learning effect was found. Those that engaged in more versus average exploratory behavior showed enhanced spatial learning evidenced by improved directional heading on Day 3 ($\bar{X}$ = 17.97 ± 3.3 vs 43.69 ± 5.7 degrees error, p<.001) and Day 4 ($\bar{X}$ = 16.43 ± 3.0 vs 36.65 ± 6.8 degrees error, p = .01, respectively). No differences were noted in swim latency. However, all lesioned animals remained impaired compared to controls (p<.05). In addition, no significant difference were seen in control animals when categorized by degree of exploration. We conclude that female rats with unilateral HPC lesions actively explore new places but that these animals need a greater than average amount of exploratory activity to improve spatial learning performance.

762.20

ACUTE CORTICOSTERONE REPLACEMENT THREE-MONTHS AFTER ADRENALECTOMY IMPROVES PERFORMANCE IN A MORRIS WATER MAZE DESPITE DEGENERATION IN THE DENTATE GYRUS. C.M. McCormick, M. McNamara, J.E. Kelsey, & N.W. Kleckner*. Departments of Psychology and Biology, Bates College, Lewiston, ME 04240, USA.

Adrenalectomy (ADX) causes loss of dentate granule cells and impairs spatial memory performance (e.g., Sloviter et al, 1989; Conrad & Roy, 1993). These losses are prevented by *continuous* replacement of corticosterone (CORT). We investigated the effects of *acute* CORT replacement on water maze performance following long-term ADX. Of the 27 ADX, 9 were considered complete ADX on the basis of plasma CORT levels, low body weight, and saline intake. The remaining were considered incomplete ADX (INC). 18 rats had sham surgery. Approximately half of ADX, INC, and SHAM groups were given CORT in their drinking water (20 μg/ml) 5 days prior to, and during the 5 days of testing. Testing consisted of animals learning to find a submerged platform ina water maze from different start locations (6 trial/day for 5 days). There was an effect of group, and an interaction between group and replacement for both latency and distance to reach the platform: ADX showed poorer performance than both INC and SHAM, who did not differ. There was no effect of CORT replacement in INC and SHAM. CORT replacement improved ADX performance compared to ADX w/o replacement, and made their performance similar to that of the other four groups. At the end of behavioural testing, ADX+CORT rats showed significant weight gain compared to all other groups. There was no effect of CORT replacement on plasma glucose levels: both groups of ADX showed lower levels than SHAM and INC. Preliminary analysis indicated a 40% reduction in area of the dentate granule layer in ADX and ADX+CORT compared to INC and SHAM. The means by which acute CORT treatment improved the performance of ADX rats is unknown.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS XIV

763.1

INTERACTIONS BETWEEN BENZODIAZEPINE AND DOPAMINE RECEPTORS IN THE MODULATION OF CORTICAL ACETYLCHOLINE RELEASE H. Moore*, M. Sarter and J.P. Bruno, Dept. of Psychology and Neuroscience Program, The Ohio State University, Columbus, OH 43210

Benzodiazepine receptor (BZD-R) inverse agonists have been shown to disinhibit cortical acetylcholine (ACh) efflux via their ability to negatively modulate GABA-mediated chloride flux in the basal forebrain. In the present experiments, the BZD-R partial inverse agonist, FG 7142, was used to test the hypothesis that dopamine (DA) receptors in the nucleus accumbens (NA) interact with $GABA_A$/BZD-R in the regulation of cortical ACh release. Systemic administration of FG 7142 was preceded by systemic or intra-accumbens administration of selective D1 (SCH 23390) and D2 (clebopride) antagonists, and the neuroleptic haloperidol. ACh efflux in the frontal cortex was measured in awake animals using in vivo microdialysis. FG 7142 (8.0 mg/kg) increased cortical ACh efflux. This increase was dose-dependently attenuated by systemic administration of the D1 antagonist (0.1, 0.3 mg/kg) or haloperidol (0.15, 0.9 mg/kg). The D2 antagonist, clebopride (10 mg/kg), however, had no effect on stimulated ACh efflux. Haloperidol administered into the NA (10, 100 μM) appeared to block FG 7142-stimulated cortical ACh efflux, whereas equimolar doses of SCH 23390 into the NA did not. Neither drug administered intracortically, via the microdialysis probe, blocked FG 7142-increased ACh efflux. These results suggest that the well known ability of FG 7142 to stimulate DA release may contribute to the induction of cortical ACh release. This DA-mediated effect does not occur locally within the frontal cortex and the site of action of D1 antagonists (i.e. non-accumbens) and neuroleptics (i.e. accumbens) can be dissociated.

763.2

BIDIRECTIONAL TRANS-SYNAPTIC MODULATION OF CORTICAL ACETYLCHOLINE IN INTACT AND 192 IgG-SAPORIN-LESIONED RATS. J.Fadel*, H. Moore, B. Givens, M. Sarter and J.P. Bruno, Dept. of Psychology and Neuroscience Program, The Ohio State University, Columbus, OH 43210

Loss of cortically-projecting basal forebrain cholinergic neurons correlates with the cognitive decline associated with Alzheimer's disease. Trans-synaptic modulation of the remaining cholinergic neurons may prove therapeutic by augmenting cortical acetylcholine (ACh) release in close temporal contiguity with the activating demands of cognitive processing. Ligands that modulate the $GABA_A$/benzodiazepine receptor (BZD-R) complex represent a putative site for this type of modulation. The present study utilized in vivo microdialysis in awake rats to assess the effects of positive and negative modulators of GABA-mediated chloride flux on cortical ACh efflux in awake intact rats and in rats sustaining cholinergic deafferentation with cortical microinfusions of the immunotoxin 192 IgG-saporin. Lesioned animals exhibited a 50-60% reduction in basal cortical ACh efflux but responded to the GABA negative modulator, BZD-R partial inverse agonist FG 7142 (8.0 mg/kg) with a stimulation of ACh efflux similar in magnitude to that seen in intact rats (100-200% above baseline). These findings were confirmed with another negative modulator of GABA, the BZD-R weak inverse agonist ZK 93 426. Acute systemic administration of the postive GABA modulator ethanol (1.0 g/kg, ip) produced an initial increase, followed by a decrease in cortical ACh efflux in intact animals. The effects of localized perfusions of ethanol and its affect on cortically deafferented rats will also be discussed.

763.3

EFFECTS OF LESIONS OF THE DORSAL NORADRENERGIC BUNDLE ON BEHAVIORAL VIGILANCE. S. Ruland*, V. Ronis, J.P. Bruno and M. Sarter. Department of Psychology & Neuroscience Program, The Ohio State Univ., Columbus, OH 43210.

The different cognitive functions of the major cortical afferent systems have remained unsettled. An operant procedure that required the animals to detect visual signals (presented for 25 - 500 msec) and to discriminate signals from non-signals was used to assess the role of forebrain noradrenergic afferents in behavioral vigilance. Measures of performance include the relative number of hits and misses (correct and incorrect responses to signals), and correct rejections and false alarms (correct and incorrect responses to nonsignals). Performance was characterized by a signal length-dependent ability to detect signals, and by performance decrements over time (vigilance decrement). Following the pretreatment with Pargyline (50 mg/kg), 6-hydroxydopamine was injected into the dorsal noradrenergic bundle (2 μg/μl/hemisphere). The lesion reduced the concentrations of noradrenaline in the cortex by > 90 % and in the hippocampus > 80 %. Postsurgery performance of the lesioned animals was characterized by relatively small and rapidly recovering decreases in both the relative number of hits and correct rejections. This finding contrasts with the persistent, potent, and selective decrease in the relative number of hits following 192 IgG-saporin-induced basal forebrain lesions (McGaughy et al. 1995). Thus, behavioral vigilance as measured by this procedure critically depends on the integrity of forebrain cholinergic, but not noradrenergic inputs.

763.4

FUNCTIONS OF CHOLINERGIC INPUTS TO VISUAL CORTICAL AREAS: EFFECTS OF VISUAL CORTICAL CHOLINERGIC DEAFFERENTATION ON VISUAL ATTENTION IN RATS. L.A. Holley* and M. Sarter. Department of Psychology & Neuroscience Program, The Ohio State Univ., Columbus, OH 43210.

The functions of the cholinergic inputs to visual cortical areas remain largely unknown. Likewise, the extent to which the loss of cholinergic inputs to the visual cortex contributes to the impairments in visual and cognitive functions in senile dementia is not clear. Our previous research on the effects of infusions of 192 IgG-saporin into the visual cortex revealed an impairment in the acquisition, but not performance, of complex visual discriminations of variable difficulty. In the present experiment, 192 IgG-saporin (0.008 μg/0.8 μl) was infused into the primary and secondary visual cortex (two infusion sites per hemisphere) of rats trained in a visual sustained attention task (McGaughy & Sarter; Psychopharmacol. 117:340-357). This task requires the animals to detect visual signals presented for 25, 50 or 500 msec and to discriminate these signals from non-signals. Present data suggest that the partial loss of cholinergic input to visual cortical areas impairs performance, primarily by decreasing the animals' ability to detect longest signals and to correctly reject non-signal trials. These findings contrast with the selective and signal length-dependent decrease in the detection of signals in animals with 192 IgG-saporin lesions of the basal forebrain and suggest a discrete function of visual cortical ACh in visual attention.

763.5

INTERACTIONS BETWEEN THE ATTENTIONAL EFFECTS OF INTRABASALIS INFUSIONS OF BZR AGONISTS AND INVERSE AGONISTS AND SYSTEMIC HALOPERIDOL. J. McGaughy* and M. Sarter. Dept. Psychology & Neuroscience Program, The Ohio State Univ., Columbus, OH 43210.

Previous work demonstrated that infusions of the benzodiazepine receptor (BZR) agonist chlordiazepoxide (CDP) or the BZR inverse agonist β-carboline β-CCM into the substantia innominata of the basal forebrain produced dissociable effects on the performance of animals tested in a behavioral vigilance task. Specifically, CDP selectively decreased the animals' ability to detect signals while their ability to correctly reject non-signal trials remained unaffected. In contrast, infusions of β-CCM selectively increased the relative number of false alarms (i.e., claims for hits in response to non-signal trials; Holley et al., Psychopharmacol., in press.). Because these findings were related to the contrasting effects of BZR agonists and inverse agonists on cortical acetylcholine (ACh) efflux (Moore, Sarter, & Bruno, Neurosci. Lett., in press), and because of the transsynaptic regulation of cortical ACh efflux by accumbens dopamine via its effects on the accumbens GABAergic projection to the basal forebrain neurons, this experiment tested the hypothesis that systemic co-administration of haloperidol (0.001 - 0.01 mg/kg) augments the attentional effects of intrabasalis infusions of the BZR agonist (40 μg/hemisphere) and attenuates the attentional effects of intrabasalis infusions of the BZR inverse agonist (3 μg/hemisphere).

763.6

CORTICAL ACETYLCHOLINE EFFLUX AND SINGLE UNIT ACTIVITY IN RATS PERFORMING OPERANT PROCEDURES ASSESSING BEHAVIORAL VIGILANCE OR SENSORIMOTOR AND MOTIVATIONAL TASK COMPONENTS. A.-M. Himmelheber, H. Moore, J. McGaughy, B. Givens, J.P. Bruno and M. Sarter*. Department of Psychology & Neuroscience Program, The Ohio State Univ., Columbus, OH 43210.

The study of the cognitive functions of cortical acetylcholine (ACh) requires experimental approaches aimed at measuring the activity of cortical cholinergic afferents in relationship to cognitive activities which recruit this system. Animals were trained in an operant vigilance task (McGaughy et al., Psychopharmacol. 117:340-357). Animals were prepared for the measurement of cortical ACh efflux as described in Moore et al. (Brain Res. 627:267-274) or for multiple single unit recording (Givens, Neurosci. in press). Medial prefrontal (i.e., prelimbic) ACh efflux increased as a result of transfer of the animals to the test chamber and task onset. Presentation of "background noise" (houselight flashing at 0.5 Hz) during one of the 4 blocks of trials significantly impaired performance, increased cortical ACh efflux and increased spontaneous prelimbic neuronal activity. Different groups of animals were trained in a series of behavioral control procedures assessing the role of motor performance, response habits, reward loss, and sensory stimuli in ACh efflux and neuronal activity. The results from these experiments provide the basis for a discussion about the procedural and conceptual issues important for studies on the cognitive functions of cortical ACh.

763.7

A CENTRAL CHOLINERGIC LINK IN THE CARDIOVASCULAR EFFECTS OF THE BZR PARTIAL INVERSE AGONIST FG 7142. V. Ronis, S. Ruland, S. Hart, M. Sarter and G.G. Berntson*. Dept. Psychology & Neuroscience Program, The Ohio State Univ., Columbus, OH 43210.

Previous work demonstrated that systemic administration of the benzodiazepine receptor (BZR) partial inverse agonist ß-carboline FG 7142 (FG) augments the cardiovascular response to nonsignal stimuli, and mimics the cardiovascular effects of a conditioned stimulus for an aversive event. Analyses of the parasympathetic and sympathetic contributions to the effects of FG prompted the hypothesis that increases in central cholinergic activity mediates the potentiation of the cardioacceleratory response by FG. Consistent with this hypothesis, the present experiments demonstrate: a) ICV infusion of the muscarinic receptor agonist carbachol mimics the response-potentiating effects of FG; b) this effect of carbachol was blocked by ICV co-administration of the muscarinic antagonist atropine; c) ICV infusions of atropine blocked the potentiation of the cardioacceleratory response by systemically administered FG, but did not alter the basal response to the stimulus; and d) 192 IgG-saporin-induced lesions of basal forebrain cholinergic neurons prevented the FG-induced potentiation of the cardioacceleratory response, without altering the basal cardiac response. These data strongly support the hypothesis that the effects of FG on cardiac reactivity are mediated via an activation of central muscarinic cholinergic mechanisms.

763.8

COMPARISON BETWEEN THE EFFECTS OF INFUSIONS OF 192 IgG-SAPORIN INTO THE BASAL FOREBRAIN OR THE CORTEX ON BEHAVIORAL VIGILANCE. D. Wendelin, J. McGaughy, B.H. Smith* and M. Sarter. Department of Psychology & Neuroscience Program, The Ohio State Univ., Columbus, OH 43210.

Cortical cholinergic deafferentation was produced either by bilateral infusion of 192 IgG-saporin into the basal forebrain (0.42 mg/ml; 0.5 μl/hemisphere) or by infusions of the immunotoxin into the cortex (a total of 5 infusions/hemisphere; 0.01 mg/ml; 0.5 μl/infusion; see Holley et al., Brain Res. 663:277-286). Following surgery, control animals and lesioned animals were retrained in an operant procedure designed to measure the animals' ability to detect visual signals (presented for 25 - 500 msec) and to discriminate signals from non-signals, i.e., behavioral vigilance. Additionally, the hypothesis that administration of the benzodiazepine receptor partial inverse agonist FG 7142 or the cholinesterase inhibitor physostigmine improves the lesioned animals' performance was tested. Basal forebrain infusions resulted in > 90 % loss of frontoparietal cortical AChE-positive fibers. Intracortical infusions decreased fiber counts by less than 50 %. Basal forebrain lesions resulted in a persistent impairment in the animals' ability to detect signals but did not affect their ability to correctly reject non-signal trials. Beneficial drug effects were not found in basal forebrain lesioned animals, possibly because of the almost complete loss of cholinergic neurons. These findings are compared with the data from the intracortically infused animals, and the usefulness of the two models will be discussed.

763.9

CORTICAL ACETYLCHOLINE AND PROCESSING CAPACITY: EFFECTS OF CORTICAL CHOLINERGIC DEAFFERENTATION ON CROSSMODAL DIVIDED ATTENTION IN RATS. J. Turchi, J.P. Bruno* and M. Sarter. Department of Psychology & Neuroscience Program, The Ohio State Univ., Columbus, OH 43210.

Divided attention is a psychological construct that is characterized by assumptions about the fixed finite capacity of subjects to simultaneously process multiple sets of information. While the role of cortical acetylcholine (ACh) in other aspects of attention (e.g., sustained, selective) is intensely studied, the extent to which cortical cholinergic inputs mediate processing capacity has remained unclear. An operant analogue of the crossmodal divided attention paradigm was developed and the potency of scopolamine to augment speed-accuracy tradeoffs was previously demonstrated (McGaughy, Turchi, Sarter, Psychopharmacol. 115:213-220). In the present experiments, local or global cortical cholinergic deafferentation was achieved by infusions of 192 IgG-saporin into the medial prefrontal or frontoparietal cortex (local deafferentation; Holley et al., Brain Res. 663:277-286) or into the basal forebrain (global deafferentation). Performance following these lesions suggests that local, restricted loss of cortical cholinergic inputs does not robustly affect the animals' ability to divide their attention between the processing of auditory and visual response rules, contrasting with the effects of global deafferentation. Cortical cholinergic mediation of processing capacity may follow mass-action rules rather than being critically dependent on single areas.

763.10

EFFECTS OF NERVE GROWTH FACTOR ON RATS GIVEN AN IMMUNOTOXIC LESION OF p75-BEARING CHOLINERGIC BASAL FOREBRAIN NEURONS. J.J. Waite*, J. Winkler, R.G.Wiley, D.A. Lappi, & L.J. Thal Dept. of Neurosciences & Neurology, UCSD & VAMC, San Diego, CA 92161.

Immunotoxic lesion provides a more selective model for testing NGF effects on the cholinergic basal forebrain. Rats received i.c.v. PBS or 192 IgG-saporin to produce either 50% (1μg) or 80% (2.7μg) overall depletions of ChAT activity in four cortical regions, hippocampus and olfactory bulbs. Half of each group was then treated i.c.v. with either 5μg/day NGF or cytochrome c for 7 weeks. Behavioral testing was performed in treatment weeks 5-7. In each brain region examined, NGF produced the greatest increase in ChAT activity in the unlesioned group and the least increase in the highly lesioned group. NGF-treated animals showed a deficit in single-trial passive avoidance retention and an increase in acoustic startle response associated with decreased habituation to startle. No lesion effect was observed in either test. In the water maze, highly lesioned rats had a longer latency to reach a visible platform. There was a significant overall effect of lesion and of NGF treatment, but no interaction. Highly lesioned rats were significantly impaired in finding a hidden platform after learning to escape to a visible platform compared to controls. NGF treatment significantly mitigated this lesion effect. All groups learned to escape to the hidden platform. This study found that NGF affected passive avoidance retention and acoustic startle independent of the cholinergic lesion. Furthermore, NGF partially improved a performance deficit produced by this lesion in the water maze.

763.11

CHOLINE EFFECTS ON MAZE PERFORMANCE ARE MODERATED BY SEX, AGE & ACH AVAILABILITY. K. Schultz*, L. Francis & N. Ward, Psychology Department, University of Winnipeg, Winnipeg, MB, Canada R3B 2E9

Allocentric performance in the Morris water maze is known to vary with sex. Males generally are slightly more efficient at this task than are females. Further, performance is also enhanced by pre- and postnatal choline supplementation. This study examined the allocentric performance of 160 Long-Evans rats, 80 of which had been exposed to enhanced choline levels throughout gestation and for 21 days after birth. Equal numbers of choline treated and control male and female rats were tested at four developmental stages: pup, prepuberty, midpuberty and adult. Finally half of the animals in each condition were tested following a scopolamine challenge. Overall, animals exposed to supplemental choline had shorter escape latencies. This enhanced performance was most apparent in male pups and prepubertal females. These differences are related to the structure of the dentate gyrus. Following scopolamine administration, animals previously exposed to choline had longer escape latencies than controls, thus choline availability as well as dentate gyrus structure differ in these groups.

763.12

INJECTION OF 192-IgG-SAPORIN INTO THE MEDIAL SEPTAL AREA EXACERBATES A SPATIAL MEMORY DEFICIT IN AGED RATS: A.W. Bannon*, P. Curzon, K.L. Gunther, and M.W. Decker, Neuroscience Research, D47W, 100 Abbott Park Rd., Pharmaceutical Discovery, Abbott Park, IL 60064-3500.

192-IgG-Saporin (IgG-Sap) is an immunotoxin that can be utilized to selectively destroy basal forebrain cholinergic neurons. In this study, IgG-Sap or PBS was injected into the medial septal (MS) area of mature (6 mo old) and aged (24-26 mo old) male Long-Evans rats. Ten days following surgery, testing began in a battery of behavioral tasks including two versions of the Morris water maze (i.e., invisible platform [Inv-P] task and 2-platform [2-p] task), inhibitory avoidance (IA), and pre-pulse inhibition of acoustic startle (PPI). At the termination of the study, tissue from several CNS sites was taken for analysis of ChAT activity.

On the Inv-P water maze task, we observed an age-associated impairment during acquisition, but no effect of IgG-Sap in either age group. Data from a probe trial conducted following 7 days of training (4 trials/day) revealed a significant spatial memory deficit in the aged rats. Interestingly, this deficit was significantly more pronounced in aged rats treated with IgG-Sap, an effect not observed in the 6 mo old rats. In the 2-P water maze task, a minimal age-associated impairment was observed. For the IA task, no significant age or treatment effect was found. In the PPI task, aged rats were significantly less responsive to the acoustic stimulus than the younger animals, but no effect of IgG-Sap was observed for either age. Finally, significant reductions (i.e., 65%) in ChAT activity of the dorsal hippocampus were observed in IgG-Sap treated rats of both ages. In conclusion, although IgG-Sap produced similar reductions in ChAT activity of dorsal hippocampus in both mature and aged rats, a significant effect of the IgG-Sap on spatial memory was only observed in aged rats.

763.13

SUBCELLULAR LOCALIZATION OF A LEARNING ASSOCIATED PROTEIN IN RAT ASTROCYTES. A. Favit*, ^M. Grimaldi, T.J. Nelson, ^G. Schettini and D.L. Alkon. Lab. of Adapt. Sys., NINDS, NIH, Bethesda, MD 20892 U.S.A.; and ^Dip. Neurosci., Sez. Farm., Univ. Napoli "Federico II", Via S. Pansini 5, I-80131 Napoli, Italy.

In this study we have analyzed the subcellular localization of cp20, a small GTP-binding protein, whose phosphorylation by PKC has been implicated in associative learning of several species. Cp20 subcellular distribution was studied in purified rat cortical type I astrocytes by indirect immunofluorescence using a polyclonal primary antibody affinity purified. Western blot analysis showed that this antibody specifically reacted with cp20 in the rat astrocytes. Immunofluorescence studies demonstrated that cultured astrocytes exhibited high cp20 immunoreactivity within the nucleus. In addition, optical sections of astrocyte nuclei studied with a confocal microscope, confirmed the nuclear localization of cp20 immunoreactivity in the astrocytes. Although cp20 has been shown to be closely related to ARF-class GTP-binding proteins, such as Sar1p, we tested whether other small GTP-binding proteins have the same subcellular localization. Surprisingly, no immunoreactivity was observed in the nuclei of rat astrocytes following immunostaining with an antibody recognizing Sar1p protein, suggesting a peculiar role for cp20 in rat cortical astrocytes. Since cp20 is specifically phosphorylated by PKC we tested the effects of phorbol esters upon subcellular localization of cp20. In serum-deprived astrocytes treated with 100 nM phorbol mystrate acetate (PMA) for a period of time ranging between 15 and 180 min, cp20 nuclear immunoreactivity was no longer detectable from the nuclei of the astrocytes. However, in the presence of serum, time-dependent treatments with PMA did not affect the immunostaining for cp20. Accordingly with our previous observations, these findings suggests that cp20 may play a role in the control of gene transcription, and moreover that PKC may exert a thight control of cp20 function in rat cortical astrocytes.

763.14

DEVELOPMENTAL INCREASE IN THE BEHAVIORAL INDUCTION OF FOS PROTEIN IMMUNOREACTIVITY IN AREA CA1 OF THE RAT HIPPOCAMPUS. Nicholas S. Waters*, Anna Y. Klintsova, and Thomas C. Foster, Department of Psychology, University of Virginia, Charlottesville, VA 22903.

Juvenile rats are unable to perform a number of learning and memory tasks which, in adults, appear to require an intact hippocampus. This inability appears despite competent sensory-motor systems, and the ability to perform other learning tasks. Hippocampal responsiveness following exposure to a memory task was studied in juvenile rats, using immunocytochemistry to FOS. FOS protein is the product of the immediate early gene *c-fos*, and has been considered a marker of cellular activity. Thirty-three littermate pairs served as experimental and control animals, and were tested at P16, P23, and P30 (birth=P0). Experimental animals were tested for 5 min in a water-motivated alternation (Y-maze) task, placed into a novel environment for 55 min, then perfused. Controls were sacrificed immediately after removal from the home cage. Immunocytochemistry for FOS protein was performed on free floating, 35 μm sections of the dorsal hippocampus. Stained sections were digitized using an Olympus Cue-2 densitometry system, and cell counts were performed in 5 regions in the hippocampus and dentate gyrus. There was no effect of age on baseline FOS expression in any region. The behavioral manipulation resulted in a significant increase in FOS immunoreactivity only in P30 subjects, and this effect was restricted to area CA1. This increase coincided with a significant increase in alternation behavior at the same age. These findings suggest that developmental changes in CA1 are related to the emergence of spatial alternation behavior, and are consistent with previous findings of presynaptic changes in the CA3-CA1 synapse at this age. This work was supported, in part, by NIH Grants NS31830 to TCF, and HD07323.

763.15

STIMULUS NOVELTY EFFECTS NACL TASTE-INDUCED EXPRESSION OF C-*FOS* IN THE CNS OF GOLDEN HAMSTERS. M. A. Barry*, E. J. Chesler, Dept. of BioStructure and Function, University of Connecticut Health Center, Farmington, CT 06030-3705.

We are using the expression of the immediate early gene, c-*fos*, as a marker for neuronal activity and protein synthesis induced by gustatory stimulation. We first showed that a series of presentations of 0.1 M sucrose or 0.15 M NaCl resulted in stimulus familiarity in golden hamsters by utilizing the phenomenon of learned harmlessness in a conditioned taste aversion (CTA) paradigm. Hamsters with preexposure to the unconditioned stimulus showed a significantly reduced aversion to the stimulus following a CTA relative to those preexposed to water. Learned harmlessness had not been previously demonstrated in hamsters. For studies of c-*fos* expression, animals were either preexposed to NaCl (familiar group), or water (novel group). The hamsters were then water deprived and given a drink of 0.15 M NaCl. After two hours the animals were sacrificed, and their brains were processed to reveal c-*fos* protein with immunohistochemistry. There was greater c-*fos* expression in the novel than the familiar group in many (but not all) areas of the brain including gustatory parts of the parabrachial nucleus and insular cortex. Thus a formed memory for familiar stimuli effects c-*fos* expression associated with gustatory stimuli even in brainstem gustatory nuclei. **Supported by 5P01-DC00168**

763.16

IMMEDIATE EARLY GENE (IEG) EXPRESSION DURING A DISCRIMINATION TASK IN ADULT CAT VISUAL CORTEX : AN IMMUNOCYTOCHEMICAL STUDY. E. Van der Gucht[1,2], L. Arckens[1,2], E. Vandenbussche[2], G.A. Orban[2] and F. Vandesande*[1]. [1]Lab. Neuroendocrin. & Immunol. Biotechnol., [2]Lab. Neuro- & Psychofysiol., K.U.Leuven, B-3000 Leuven, Belgium.

Using immunocytochemical methods we investigated the influence of a visual learning process on the expression of two IEGs, c-fos and zif-268, in the primary visual cortex of the adult cat. We compared the expression levels of both IEGs in the visual cortex and somatosensory cortex (SII) from different cats before and after being trained in a bar orientation discrimination task at different performance levels.

In the visual areas 17 and 18, but not in SII, we detected different degrees of c-fos and zif-268 labeling depending on the experimental conditions. The expression level of c-fos and zif-268 was elevated in the bar orientation discrimination cat after five days of training, during which the cat really learns to distinguish between two bars of different orientation. In contrast the expression levels of both IEGs in the brains, i.e. before orientation discrimination training, or after completion of the training (75% correct for an orientation difference of 5°) in bar orientation discrimination revealed a basal level of c-fos positive nuclei in the supra- and infragranular layers, while the expression of zif-268 immunoreactive neurons even was below basal level in layers II, III, IVc and VI of area 17 and 18 in a full-trained cat.

Our results show that a discriminative learning process induces c-fos and zif-268 expression in areas 17 and 18 of the cat visual cortex.

763.17

EFFECTS OF MATERNAL EXPERIENCE ON FOS-LIR IN MPOA, AMYGDALA AND CORTEX. A.S. Fleming* and M. Korsmit. Erindale College, University of Toronto, Mississauga, Ontario L5L 1C6.

In order to determine what brain sites are activated during the acquisition and retention of a maternal experience in postpartum rats, two studies examined the number of cells showing Fos-like immunoreactivity (Fos-lir) in brains following re-exposure to pups (USs and CSs) and pup-associated cues (CSs) in maternally-experienced and inexperienced rats. In the first study, day 1 postpartum rats were given a 2 hour interactive experience with pups and then re-exposed to pups or to a neutral stimulus (perforated box) 4 days later. In the second study, day 1 postpartum rats were given a 4 hour interactive experience with pups and a neutral box in a new cage or were left undisturbed. On day 10 postpartum animals were exposed either to pups in the perforated box in the new cage (USs and CSs), to the box and cage without pups (CSs alone), or left in the home cage. For both studies, at the end of the test phase animals were sacrificed and brains were prepared for immunohistochemical detection of Fos-lir.

In both studies the brain site showing the most consistent difference between experienced and inexperienced animals was the medial preoptic area (mpoa), an area of the brain known to be important for the expression of maternal behavior. Fos-lir was higher in the mpoa of the experienced groups. Marginal experience effects were also found in the basolateral amygdala, an amygdaloid structure involved in the formation of associations within biologically-relevant contexts. The third site showing experience effects (in the first study only, where animals actually physically interacted with pups on re-exposure) was the parietal cortex. In a fourth site, the prefrontal cortex, experienced animals showed lower Fos-lir than did inexperienced animals, an effect that may reflect exposure-based habituation to novelty of the pup stimuli.

Supported by MRC Grant to A.S. Fleming

763.18

REF-1 PROTEIN EXPRESSION FOLLOWING FEAR CONDITIONING. O. Stiedl*[1], S. Milanovic[1,2], O. Laban[1], and J. Spiess[1]. [1] Dept. of Molecular Neuroendocrinology, Max-Planck-Institute for Experimental Medicine, Hermann-Rein-Str. 3, D-37075 Goettingen, Germany; [2] Central Institute for Mental Health, J5, D-68159 Mannheim, Germany

Male mice (C57/BL6J) were subjected to fear conditioning in order to investigate specific changes in the expression of *ref-1* protein. Freezing frequency and locomotor activity were evaluated as behavioral parameters. Heart rate was monitored to assess stress levels. In the training phase animals were placed for 3 min into a conditioning box, then presented with a pulsed (5 Hz) tone stimulus (1 kHz, 80 dB, 30 s) before a foot shock (2 s, 0.7 mA) was applied. Animals were tested the day after training either for contextual retention by placing them into the same box for 6 min without auditory or foot shock stimulation or for tone-dependent retention in an altered context. In addition, unpaired conditioning was performed to determine the retention-specificity of *ref-1* protein expression.

Ref-1 is a nuclear protein controlling the activity of homo- and heterodimers of immediate early genes (*c-fos, c-jun*) by redox activation and also functions as a apurinic/apyrimidinic DNA repair enzyme. *Ref-1* protein could be an important molecular signal in the proposed cascade from ion channel activation to learning and memory.

In brains of control mice, *ref-1* protein was highly expressed in the hippocampus (CA1, DG), frontal and parietal cortical areas, and in nc. septalis lateralis and medialis. A time-dependent differential expression of *ref-1* protein was observed in the time range from 0-180 min following the training phase with a peak difference at 60 min. At that point *ref-1* protein expression was absent in the hippocampus and strongly increased in the 2. and 3. layer of the parietal cortex. After 180 min the *ref-1* protein expression pattern was identical with the one in control animals. Differential expression pattern of *ref-1* protein following contextual and tone-dependent retention will be discussed.

763.19

ANALYSIS OF MEMORY CONSOLIDATION USING INTRACEREBRAL INFUSIONS OF ANTISENSE OLIGONUCLEOTIDES TO THE TRANSCRIPTION FACTOR CREB. J.F. Guzowski, B. Setlow, G.D. Novack* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory, and Department of Psychobiology, University of California, Irvine, CA 92717-3800.

The regulatory transcription factor CREB is constituitively expressed in the brain and can be phosphorylated both by PKA and CaMK, which are activated by cAMP and Ca^{2+}, respectively. Phosphorylated CREB activates transcription of genes containing cyclic AMP response elements (CREs) in their promoters. Thus, CREB and related transcription factors provide the link between changes in second messenger levels to changes in the cell's transcriptional program. Recent publications have shown a role for CREB in long-term memory consolidation. The transgenic approaches used in these studies, however, do not allow anatomical localization of the observed effects to defined structures. To gain a greater understanding of the role of CREB in the consolidation of different tasks in the mammalian brain, we have initiated studies in which antisense CREB oligonucleotides are stereotactically infused into defined regions of the rat brain and the effect on learning and memory are compared to buffer and mismatch oligonucleotide control groups. The hippocampus and the amygdaloid complex have been chosen for study because of their well documented role in the learning and memory of different tasks. Tasks chosen for study are the hidden platform water maze and continuous multiple trial inhibitory avoidance (CMIA). Preliminary results indicate that CREB antisense oligonucleotides infused into the hippocampus impair memory consolidation but not acquisition in both tasks. Additional preliminary evidence suggests that CREB antisense oligonucleotides infused into the amygdala do not affect memory consolidation in CMIA. These results suggest that hippocampal, but not amygdaloid, CREB mediates changes in transcription important for memory consolidation.

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763.20

CREB LEVELS ARE REDUCED IN THE RAT HIPPOCAMPUS FOLLOWING OVARIECTOMY AND HYPOGLYCEMIA-INDUCED SEIZURE. K. S. Panickar[1,3,*], K. R. Purushotham[2], G .Rajakumar[3], M. A. King[4] and J .W. Simpkins[3]. [1]Dept. of Pharmacology & Experimental Therapeutics, [2]Dept. of Oral Biology, [3]Center for Neurobiology of Aging and Dept. of Pharmacodynamics, [4]Dept. of Neuroscience. University of Florida, Gainesville, FL 32610..

cAMP response-element binding protein (CREB) is a transcription factor which has been implicated in the activation of protein synthesis required for long-term memory. Previous research from our laboratory has shown that memory is compromised following ovariectomy and that some of these memory deficits can be ameliorated with estrogen replacement. We have also reported cognitive deficits following severe hypoglycemic episodes in rats. Given that CREB is critical for memory, we investigated the levels of CREB in rat hippocampus in these two models of memory dysfunction. In Experiment 1, immunoblot analysis of proteins (SDS-PAGE) using polyclonal antibody to CREB revealed that CREB had decreased by 23% in the hippocampus following one week of ovariectomy while the estrogen-replaced animals showed a recovery in CREB levels compared to ovariectomized animals. This suggests that estrogen treatment may be beneficial in preventing the decrease of CREB in rats, thereby playing a role in memory consolidation. In Experiment 2, we found that insulin-induced seizure reduced CREB by 55% compared to controls in the hippocampus. Immunohistochemistry also revealed a decrease number of CREB positive cells in CA1, CA3, dentate gyrus, and the entorhinal cortex. Taken together these results suggest that CREB is an important mediating factor in memory loss following ovariectomy and severe hypoglycemic episodes. (Supported by NIA AG 10 485 to JWS).

763.21

GLUCOSE PREFERENCE AND RECOGNITION OF PERIPHERAL GLUCOSE UTILIZATION IN THE BRAIN OF RATS WITH INSULIN DEPENDENT DIABETES MELLITUS BY A FUNCTIONAL MRI AFTER INSULIN TREATMENT. K.Torii*[1,2], M. Mori[1], T.Yokawa[1], T.Murata[1], K.Tajima[1], M.Takezawa[1] and T.Ono[3]. [1]Torii Nutrient-stasis Project, ERATO, R&D Corp. of Japan, Yokohama 221, [2]Ajinomoto Co. Inc., Central Res. Lab., Yokohama 244, and Toyama Med.&Pharmaceu.Univ., Toyama, 930-01, Japan.

The patients with diabetes mellitus generally display a strong preference for sweets as energy sources to compensate for disorder of peripheral glucose utilization by failure of insulin function. Either preference for glucose or change of the brain function treated with insulin was examined before and after Streptozotocin (STZ) administration to Sprague-Dawley male adult rats. Preference for glucose in rats was evoked several hours after STZ treatment (50 mg/kg BW, i.p.) and reached a plateau level for a week. Histological findings of the pancreas also supported these behavioral changes as an insulin dependent diabetes mellitus (IDDM). Each anesthetized similar rat was settled in the 40 cm bore of a 4.7 tesla MRI. Fasted glucose in plasma was above 400 mg / dl and normalized below 80 mg/dl at 100 min after insulin injection (20 U/kgBW, i.p.). The brain O_2 consumption was monitored by T2* weighted intensity and visualized chronologically by rapid gradient echo pulse sequence method. The intensity changed in the hippocampus at 20-30 min, the paraventricular nucleus at 30 min, the thalamus and the dorsomedial hypothalamus at 40-50 min and then the ventromedial hypothalamus at 100 min after insulin injection. These changes were recovered at 120 min by normal glucose utilization with insulin treatment. Saline treatment in IDDM rats as control never caused any intensity change in the brain. The degree of glucose preference and chronological brain functional changes by MRI can be a useful diagnosis for status of diabetes mellitus.

764.1

DISHABITUATION OF TAIL PINCH-INDUCED GLUTAMATE AND LACTATE RELEASE BY LOCAL PERFUSION OF RECEPTOR AGONISTS IN RAT PREFRONTAL CORTEX. M.Takita*. Nervous Informatics Lab., Biosignalling Dept., Natnl. Inst. of Biosci. and Human-Technol., Ibaraki 305, Japan.

To clarify the roles of rat medial frontal cortex (mpFC) responding to stressful stimuli, we have been studying the habituation of stressful stimuli-induced glutamate (GLU) and lactate (LAC) release by using continuous *in vivo* brain microdialysis with less than 1 min-resolution. In brief, the dialysate was mixed directly with an assay solution in a T-tube and GLU or LAC was measured in a flow cell under the freely moving condition. We have reported the following. 1) The mpFC GR was increase immediately and transiently from its steady state level after1min tail pinch (TP). The mpFC LR was also increased by various stimuli (TP, 5min 100dB white noise, and 5min immobilization). 2) The 2nd response to the same stimuli given 1 hour later was smaller than the 1st response (about 35% decrease). 3) GR and LR both showed a decreasing response in septum. 4) The mpFC GR response occurred under reserpinization while the LR was completely abolished. In addition, we found that the habituation of TP-induced GLU or LAC release disappeared by perfusion of DA (10^{-4}M) or NMDA (10^{-4}M) into the probe for 5min between the two trials in a manner of dis-habituation. These treatments increased the 2nd TP-induced response of GLU or LAC compared with the 1st one {GLU: 133.4±11.4% (n=7), LAC: 360±255% (n=3)}. These results suggest that there are at least two different systems for information storage processing specialized in rat mpFC.

764.2

AMINO-ACID REGULATION OF STRESS-INDUCED PROENKEPHALIN GENE EXPRESSION IN THE PVN

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Proenkephalin (ENK) is expressed in the paraventricular nucleus (PVN) of the hypothalamus where it is regulated by a number of physiological stimuli. Excitatory and inhibitory amino acid neurotransmitters are present at high levels within the PVN and have putative roles in hypothalamic neuroendocrine regulation. Using a hypertonic stress paradigm in a transgenic mouse model expressing a proenkephalin-ß-galactosidase fusion gene, we have studied the effects of excitatory and inhibitory amino acids on the expression of the proenkephalin gene in the PVN under basal conditions or in response to a 1.5M saline stress. The GABA-ergic drugs aminoxoyacetic acid (AOAA, a GABA-transaminase inhibitor), GABA, or baclofen (a GABA-B agonist) all induced expression of the transgene at 6hrs following i.p. administration. Chronic (4 day) administration of AOAA inhibits both basal and stress-induced expression of the transgene. Muscimol (a GABA-A agonist) inhibits both basal and stress-induced expression of the transgene. MK-801 (a non-competitive NMDA antagonist) inhibits both basal expression and 1.5M saline stress-induced expression of the transgene. The excitatory amino acids, NMDA and kainate, do not induce proenkephalin gene expression in the PVN after acute or chronic (4 day) or administration. Alterations in transgene expression were compared with alterations in c-Fos expression in the PVN using the same paradigms *in vivo* and in hypothalamic cultures. A dissociation between transgene and c-Fos expression was seen following administration of some of these drugs. These results show that excitatory and inhibitory aminoacids can modulate basal and stress induced proenkephalin gene expression in the PVN and may be used to probe positive and negative transcriptional regulation of the proenkephalin gene in this model.

764.3

ACUTE STRESS ALTERS EXPRESSION OF IMMUNOREACTIVE INTERLEUKIN-1β IN THE MALE RAT HIPPOCAMPUS AND PARAVENTRICULAR HYPOTHALAMUS. **A. P. Ray*, N. Quan, J. M. Weiss and A. N. Clancy. Department of Psychiatry and Behavioral Sciences, Emory University Medical School, Georgia Mental Health Institute, 1256 Briarcliff Rd., Atlanta, GA 30306.**

The presence of interleukin-1β (IL-1β) in brain has been demonstrated in several recent studies. Other studies have suggested that brain IL-1β may mediate various responses to stress. This study examined stress-induced changes in IL-1β immunoreactivity in two brain areas implicated in stress response modulation: the hippocampus (HIP) and paraventricular hypothalamic nucleus (PVN). Rats, processed as matched pairs, were given either one hour of footshocks (experiment) or taken directly from their home cages (control). Brains were removed, sectioned and incubated in anti-rat IL-1β antibodies (RIL-1β Ab), anti-human IL-1β antibodies (HIL-1β Ab), or no primary antibodies (zero Ab control); sections were processed by the avidin-biotin-immunoperoxidase method. Optical density (OD) of labeling for IL-1β was measured by computer-assisted video densitometry. In the HIP pyramidal cell layer (CA1 and CA3), the OD of nonstressed rats (16.1 ± 1.93) was significantly greater (p<.04, n=9) than the OD of stressed rats (9.96 ± 1.99), while in the PVN the OD of nonstressed rats (5.58 ± 1.67) was significantly lower (p<.03, n=9) than the OD of stressed rats (12.5 ± 3.14). We conclude that, following exposure to acute stress, IL-1β was reduced in HIP pyramidal cells and increased in the PVN. This suggests that IL-1β is released in the hippocampus and is accumulated (possibly synthesized?) in PVN cells following acute stress. (Supported by NIMH Grant MH-50420).

764.4

NEUROPEPTIDE Y (NPY) GENE EXPRESSION DURING STRESS. S.P. Han*, X.L. Chen, Y.M. Wu, L. Naes and T.C. Westfall. Dept. Pharmacol. and Physiol. Sci., Saint Louis Univ. Health Sci. Ctr., St. Louis, MO 63104.

Hypertension is a multifactorial disease involving both environmental and genetic factors. A major environmental factor that has often been associated with hypertension is stress. Neuropeptide Y (NPY) is a vasoactive peptide that is co-synthesized and co-released together with catecholamines from sympathetic nerves and the adrenal medulla. Both NPY and catecholamines may be involved in mediating stress-induced increases in blood pressure and are potential links to hypertension, itself. In the present studies we have investigated changes in NPY gene expression resulting from insulin-induced hypoglycemic stress. Northern hybridization techniques were employed to detect changes in NPY mRNA abundance in the superior cervical ganglia (SCG) and the adrenal medulla. Subcutaneous injection of insulin produced dose-dependent increases in NPY mRNA abundance both in the SCG and the adrenal medulla. The hypoglycemia-induced increase in NPY gene expression in the SCG was attenuated by denervation of the ganglion. The hypoglycemia-induced increase in NPY gene expression was mimicked by direct activation of acetylcholine-nicotinic receptors produced by nicotine in a dose-dependent manner. NPY immunoreactivity (NPY-ir) was determined by radioimmunoassay. NPY-ir in the adrenal medulla was also increased following subcutaneous administration of nicotine. These results suggest that increased NPY mRNA levels during stress appear to be due to trans-synaptic activation of the post-ganglionic neurons. Increased synthesis and subsequent release of NPY may be partially responsible for the increased vascular observed during stress. (Supported by HL-26319 and HL35202)

764.5

ORAL ADMINISTRATION OF CORTICOSTERONE MIMICS EFFECTS OF STRESS ON HIPPOCAMPAL CA3c DENDRITIC STRUCTURE. A.M. Magariños*, M. Orchinik and B.S. McEwen. Lab. of Neuroendocrinology, Rockefeller University, New York, NY 10021.

The hippocampus undergoes plastic changes in response to stress. Following repeated stress, rats display deficits in spatial memory tasks and a corresponding atrophy in apical dendrites of CA3c neurons. One mediator of this structural change is corticosterone (CORT); injection of supraphysiological doses of CORT mimics the stress effect, and blockade of stress levels of CORT prevents stress-induced dendritic atrophy. In this study, we administered CORT in a non-stressful manner to isolate CORT effects from more generalized stress effects. Adrenally-intact male rats were administered either CORT (400 mg/ml) or vehicle (VEH; 2.4% EtOH) in the drinking water for 3 weeks. This protocol produced plasma CORT levels similar to that seen in stressed rats, although the normal diurnal rhythm of CORT release was disturbed. During this period, rats were either subjected to daily restraint stress (6 hrs/day) or undisturbed. At the termination of treatment, brains were processed for Golgi impregnation.The number of apical dendritic branch points in CA3c was significantly reduced in both stress/VEH- and nonstress/CORT-treated animals, relative to nonstress/VEH controls. The total apical dendritic length was also decreased, but not significantly, in both stress/VEH- and nonstress/CORT-treated animals. Interestingly, there was no tendency for a reduction in dendritic branch points or dendritic length in stress/CORT-treated rats. These data support the conclusion that physiological levels of CORT alone can induce dendritic atrophy, but also suggest that sustained exogenous CORT may modulate stress effects on hippocampal neuronal structure. Supported by MH41256 and NS07080 (B.S.M.).

764.6

VIP AND AVP mRNA EXPRESSION IN THE SUPRACHIASMATIC NUCLEUS (SCN) OF THE RAT BRAIN FOLLOWING SHOCK STRESS. L. Zhou and R. F. McGivern*. Dept. of Psychology, San Diego State Univ., San Diego, CA 91920.

Vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP) are expressed differentially in the SCN. VIP in the brain can be regulated by adrenal steroids (Rotsztejn et al, 1980) and VIP in the SCN has been suggested to regulate phase shifts in the circadian rhythm of locomotor activity of free-running rats (Albers et al, 1991). Therefore, we examined the level of AVP and VIP mRNA expression in the SCN, as well as the SON and PVN, in response to 30 minutes of stress. During the 3-4th hour of the light cycle (12:12), two or six month old male Sprague-Dawley rats were administered 15 seconds of intermittent shock (0.5 mA) every 5 minutes for 30 minutes. They were decapitated at 75 or 135 minutes after the onset of the shock-stress session. Control animals were placed in similar cages with no shock and sacrificed at the 75 minutes time point. Brains were removed and subsequently probed for AVP and VIP mRNA using *in situ* hybridization. The exonic probes were labeled with ^{35}S deoxyadenosine-5'- (-thio) triphosphate by terminal transferase enzyme. After hybridization with the probe (~1.10 x 10^6 cpm/µl), sections (16 µm) were dried and exposed to Hyperfilm B_{max} for 1 1/2 days for AVP, and for 2 1/2 days for VIP. Sections were dipped in nuclear emulsion and exposed for five and three days respectively. Results revealed a significant decrease in optical density of VIP mRNA expression in SCN at 75 and 135 min in response to the stress at both ages. In addition, there was a significant decrease in VIP with age. Analyses employing grain counts over single cells, revealed a significant decrease in the number of SCN cells expressing VIP mRNA following stress. However, no significant differences following stress were observed in AVP mRNA expression in the SCN, the SON and the PVN. Further studies have shown this stress will significantly phase advance the activity of free running animals under constant light conditions. Overall, these results indicate that stress can alter SCN gene expression and provide indirect support for the hypothesized role of VIP in regulation of phase shifts in circadian rhythms. (AA06478, RFM).

764.7

RESTRAINT STRESS AND ACUTE ETHANOL ADMINISTRATION ALTER ENDOGENOUS LEVELS OF A NEUROACTIVE STEROID. D.A. Finn*, A.J. Roberts and J.C. Crabbe. VA Medical Center and Dept. Medical Psychology, Oregon Health Sciences University, Portland, OR 97201.

The GABA-agonist neuroactive steroid 3α-hydroxy-5α-pregnan-20-one (3α,5α-P) can reach endogenous levels which potentiate the action of GABA *in vitro*, suggesting that it may be a physiologically relevant neuromodulator. With the single exception of the report that 10 min. swim stress significantly increased brain and plasma 3α,5α-P, there are no available data regarding the influence of stressors on endogenous 3α,5α-P. Therefore, we measured the time course for changes in plasma 3α,5α-P and corticosterone (CORT) following 30 min. restraint stress and ethanol injection (4 g/kg) in genetically heterogeneous male mice. Restraint stress significantly increased plasma 3α,5α-P and CORT, which was evident immediately post-restraint (t = 30 min). Plasma 3α,5α-P remained significantly elevated at 60 min, while plasma CORT remained elevated at 60 and 90 min. Both steroids had returned to basal levels at the later time points (150 and 210 min). This result contrasts with the report that swim stress-induced increase in plasma 3α,5α-P did not occur until 60 min post-stress. Acute administration of ethanol produced a significant increase in plasma CORT at 0.5, 2 and 7 hrs post-injection and a significant decrease in plasma 3α,5α-P at 7 hrs post-injection. Withdrawal severity, measured by an increase in handling-induced convulsion scores peaked at 6 hrs and remained elevated at 12 hrs post ethanol. Collectively, these findings suggest that there are differences in the stress response, measured by plasma 3α,5α-P and CORT, depending on whether swim, restraint or alcohol withdrawal was the stressor. Supported by grants NS09264 (DAF), AA08261 (JCC) and the VA (JCC)

764.8

THE EFFECTS OF MICROGRAVITY ON GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) and BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) mRNA EXPRESSION IN THE RAT BRAIN. B. W. Prahl,* J. R. Day. Dept. of Biology, The Pennsylvania State University, University

The objective of this study was to examine the effects of microgravity on neurodegeneration in rats using GFAP and (BDNF) as markers. GFAP mRNA is a useful astrocytic marker that increases in response to injury, neurodegenerative disease, and aging. BDNF plays an important role as a growth factor in several neuronal populations. The rats in this study were also treated with Bone Morphogenic Factor (BMF) as part of an experiment to test this growth factor's actions on bone density loss in microgravity. Twenty-four ovariectomized adult female Fisher 344 rats obtained from NASA through the Penn State Center for Cell Research, were housed individually and divided into four groups:1) flight/vehicle-injected, 2) flight/BMF-injected, 3) ground/vehicle-injected controls, 4) ground/BMF-injected controls. The flight animals were exposed to microgravity (PSE4) for 14 days, and at the conclusion of the flight, brains were removed and frozen for *in situ* hybridization. Grain densities per cell were determined on emulsion coated slides via computer-assisted videodensitometry for different hippocampal strata. All flight animals showed a 40% decrease in GFAP mRNA compared to ground controls. There was no significant difference in GFAP mRNA expression between flight/BMF group and ground/BMF controls. BDNF mRNA levels were not significantly different in the flight group compared to the controls. The decreased GFAP mRNA expression might be due to elevated glucocorticoid levels induced by reentry stress and/or by microgravity itself. The altered GFAP expression suggests that exposure to microgravity might alter gene expression in the brain, particularly in astrocytes.

764.9

INHIBITION OF STRESS-INDUCED C-*fos* EXPRESSION IN THE RAT FOREBRAIN BY TRANSCRANIAL ELECTROSTIMULATION (TCES). G.P. Kozlowski and V.P. Lebedev*. Dept. Physiology, Univ. of Tex. Southwestern Med. Cen., Dallas, TX 75235 and Cen. for Research, Parker College, Dallas, TX 75229.

The effect of TCES on stress-related c-*fos* expression was determined using cranial subcutaneous needle electrodes in rat brains from animals that were either restrained (intensive stress-IS) or unrestrained (mild stress-MS). Control animals were untreated with TCES. MS and IS animals were stimulated for one hour with the Neurotrans unit using parameters of stimulation (70Hz, 3.5msec, 1.2ma) previously shown to ameliorate pain in rats (Fiziol Zhurn SSSR, 74:1094, 1988). c-*fos* immunopositive nuclei in several brain regions from all animals were manually counted in a systematic, reproducible fashion using stringent criteria established by one operator and confirmed by another. The brain regions studied were: 9 areas of the cortex (layers I-IV, V, VI), hippocampus, dentate gyrus, thalamus (6 nuc), habenula, hypothalamus (7 nuclei including the supraoptic-SO & paraventricular-PA) and 2 basal ganglia. Administration of TCES significantly inhibited stress-induced c-*fos* expression in most of the areas studied except for the centromedial thalamic and perifornical nuclei which had an insignificant increase in the number of c-*fos* neuronal nuclei. For example, c-*fos* neurons decreased ≈72.3% in magnocelluar cells of PA and ≈75.0% for SO. These results in experimental animals correlate with our previous data on antistress activity of TCES in human and other animals treated for gastric ulcer repair or alcohol withdrawal (Biogenic Amines 5:427, 1988). Supported by NIAAA AA-06014.

764.10

DIFFERENTIAL INDUCTION OF FOS PROTEIN FOLLOWING TAILPINCH OF YOUNG AND OLD MALE RATS. W.J. Smith, J. Stewart, and J.G. Pfaus*. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, QC, Canada.

Tailpinch is a short-term stressor that induces a general arousal in the rat. This stimulation increases sensitivity to incentive cues, for example, making sated rats eat more, sexually sluggish male rats copulate, and can increase dopamine release in the nucleus accumbens. However, tailpinch becomes less effective with age. We were interested in examining where in the brain tailpinch might induce neural activation. Young (2 to 3 months) or old (one year) male rats were administered either 5 tailpinches (one every 2 min), 1 tailpinch, or sham treatment (n=4 per stimulation condition). Rats were sacrificed 1 hr following the onset of stimulation for histological analysis of Fos immunoreactivity. Serial coronal sections were taken from the area of the nucleus accumbens back to the central grey in each rat. Tailpinch produced a monotonic increase in Fos in a variety of brain regions, including the nucleus accumbens, striatum, lateral septum, lateral preoptic area, paraventricular hypothalamus (PVN), medial amygdala, ventral tegmental area (VTA), and central grey. A significant difference in Fos induction by age was found in the PVN and VTA, in that older rats had less Fos within these regions following both 1 and 5 tailpinches. The other areas are currently being counted and will be presented. These data indicate that tailpinch activates regions of the brain known to be involved in behavioral responses to different types of incentive cues. These data also suggest that the decrease in sensitivity to tailpinch in older animals may reflect a decrease in the ability ot this sitmulus to activate these different brain regions.

764.11

DIFFERENTIAL EXPRESSION OF FOS PROTEIN IN THE VENTRAL TEGMENTAL AREA OF COPING AND NON-COPING RATS. M.L. Coco* and J.M. Weiss. Dept. of Pharmacology, Duke Univ., Sch. of Med., Durham, NC 27710 and Dept. of Psychiatry & Behavioral Sciences, Emory Univ. Sch. of Med., Atlanta GA 30306.

We are interested in establishing the central pathways that underlie effective coping behavior. Previous studies have demonstrated the ability of Fos immunocytochemistry to detect neuronal areas of activation under a variety of conditions. Since rats able to perform a coping response in a stressful situation show different behavioral, neurochemical, and hormonal responses from rats unable to perform a coping response, we examined expression of Fos protein in the brains of coping and non-coping rats using immunocytochemical techniques. Feedback stimuli following performance of a coping response have been shown to be an important factor in establishing effective coping behavior, i.e., responses that in addition to removing a stressor also remove the negative physiological and/or behavioral effects of being exposed to the stressor. Quartets of Sprague-Dawley rats consisting of an Avoidance-escape animal able to control its shock and receiving a feedback stimulus following performance of the coping response (AE-FB), a Yoked animal receiving the same shocks and feedback but without control over them (Y-FB), a Yoked animal similar to Y-FB but without feedback (Y-NFB), and a Box-control animal placed in the same apparatus and receiving feedback but no shock (BC-FB), were given several training sessions over a period of weeks and then were exposed to a 1.5 h test session. The feedback stimulus was an 8 s compound stimulus consisting of lights off and a tone. Immediately after the test session, the rats were returned to their home cages. They were anesthetized 1 h later, followed by perfusion and fixation of their brains. A fifth, Home-cage control, rat was used to determine basal levels of Fos expression. Fos was visualized in 40 μm sections by the ABC immunoperoxidase method. Preliminary results indicate that Fos protein expression was increased in the ventral tegmental area of AE-FB rats compared to all other groups. Numbers of Fos-positive cells did not differ between AE-FB, Y-FB, and Y-NFB rats in the caudate-putamen or locus coeruleus.

764.12

EXPRESSION OF NEURONAL PLASTICITY-RELATED MARKERS IN HIPPOCAMPUS OF CONGENITAL LEARNED HELPLESS AND RESISTANT RATS UNDER CONDITIONS OF INESCAPABLE STRESS. G. A. Laforet†, E. Edwards⌖, J. A. King†, and D. T. Rivera.†* †Department of Psychiatry, Behavioral Neuroscience Division, University of Massachusetts Medical School, Worcester, MA 01655. ⌖Department of Pharmacology and Toxicology, University of Maryland, Baltimore, MD 21201.

Environmental stress requires adaptations in behavior and homeostasis to optimize survival. The central nervous system mediates stress responses not only by neurophysiological changes but also potentially by alterations in neuronal connectivity. Such changes may be particularly prominent in the hippocampus, a brain region implicated in learning and memory, and one which retains a high degree of plasticity into adulthood. This study investigates stress-related hippocampal plasticity in rats with learned helplessness, a well-studied paradigm which may model human stress-induced neuropsychiatric illness. Learned helpless rats fail to escape from avoidable shock after having been subjected first to inescapable shock. Strains of rats have been bred to foster either increased susceptibility (cLH) or resistance (cNLH) to development of learned helplessness. Hippocampal mRNA expression of plasticity markers F1/GAP 43 and SCG 10 was assessed in these animals using Northern blot analysis. A time course study of F1/GAP 43 and SCG 10 expression revealed differences in marker message levels between cLH and cNLH rats. In particular, F1/GAP 43 and SCG 10 mRNA levels were diminished 24 hours after two shock stress sessions in congenital learned helpless rats, but not in resistant animals. These results suggest that there may be genetically-based differences in patterns of central nervous system plasticity that underlie inherent vulnerability or adaptability to stress.

764.13

STRESS-INDUCED INCREASES IN REGIONAL BRAIN CONCENTRATIONS OF NEUROPEPTIDE Y-LIKE IMMUNOREATIVITY. F.B. JOLICOEUR*, P. C. SIMARD, R. LEDUC, J.P. BOULENGER, AND A. CADIEUX. Depts. of Psychiatry and Pharmacology, University of Sherbrooke, Sherbrooke, Qué., Canada, J1H 5N4.

Experimental evidence suggests an important implication of neuropeptide Y (NPY) in anxiety. For example, we have recently shown that NPY plasma concentrations were significantly higher in panic disorder patients (Society for Neuroscience abstr. 1994). To better understand the relationship between NPY and anxiety, we examined the effects of experimental stress on NPY concentrations in several brain regions in rats. Animals (male Long Evan rats, 250-275g) were separated into two groups (n=8): one control and one experimental stress. Animals were handled for 10 min. every day for four days prior to the experiment. On the fifth day, a standard restraint stress was imposed on experimental animals for one hour. Immediately after, dissection of the following regions was performed: nucleus accumbens, striatum, septum, hypothalamus, hippocampus, amygdala, frontal cortex and prefrontal cortex. NPY concentrations were measured by radioimmunoassay. Results revealed that, in comparison to control animals, NPY levels in stressed rats significantly ($p<0.05$) increased in septum (5.4 pmol/g vs 10.2 pmol/g), hippocampus (3.9 pmol/g vs 6.6 pmol/g), frontal cortex (5.9 pmol/g vs 7.8pmol/g), prefrontal cortex (5.8 pmol/g vs 10.8 pmol/g) and amygdala (4.5 pmol/g vs 15.4 pmol/g). The marked increase in the amygdala is interesting because of the well known involvement of this limbic region in anxiety. There were no significant differences between control and stressed animals in the other regions studied. The results of the present study support the hypothesis that NPY plays a functional role in anxiety. (Supported by le Reseau en Santé Mentale du Fonds de la Recherche en Santé du Québec).

764.14

THE STRESS-INDUCED POLYAMINE RESPONSE; ADAPTATION AND INHIBITION BY LITHIUM. **V.H. Gilad*, J.M. Rabey and G.M. Gilad.** Lab. of Neuroscience, Dept. of Neurology, Tel Aviv-Elias Souraski Medical Center, Tel-Aviv 64239, Israel.

As a transient increase in polyamine (PA) metabolism is a common response to stressful stimuli, our hypothesis is that a maladaptive PA response is involved in the pathophysiology of affective disorders. Previously, we found that, unlike peripheral tissues, the *acute* stress-induced PA response in the brain can be prevented by long-term (days), but not by short-term treatment with lithium. Based on the premise that *chronic* stress may best approximate stressful life events which can exacerbate affective disorders, our present findings demonstrate that in the rat brain, inescapable chronic intermittent stressor results in a recurring PA response. In contrast, in the periphery (liver), a similar stressor causes habituation of the PA response. The recurring brain PA response can also be blocked by long-term lithium treatment. Interestingly, immature rats do not show the characteristic brain PA-response. The ontogenesis of this effect is brain region-dependent. In general, the developmental switch to a mature PA response corresponds in time to the cessation of the "stress non-responsive period" in the pituitary-adrenocortical system. Our findings implicate an over-reactive PA response as a component of the adaptive, or maladaptive, brain response to stressful events, and as a novel molecular target for lithium action.

764.15

MOTOR ACTIVITY AND QUANTITATIVE AUTORADIOGRAPHIC STUDIES OF MUSCARINIC RECEPTORS IN THE BRAIN OF RATS SUBJECTED TO THE FORCED SWIMMING TEST. E. Carrizo[1], G. Cano[2], H. Suárez-Roca[1,2], E. Bonilla[1,2]*. [1]Instituto Investigaciones Clínicas, Universidad del Zulia and [2]Fundacite-Zulia, Maracaibo, Venezuela.

A cholinergic dysfunction has been involved in the neurobiological mechanisms of stress and depression. In addition, the Forced Swimming Test (FST) has been extensively used as an animal model of depression. In the present study, the distribution of muscarinic cholinergic receptors in the brain of rats subjected to the FST was determined to evaluate the cholinergic hypothesis of depression. Male Sprague-Dawley rats were subjected to the FST for 30 min daily during 15 days. The sham group was placed in the same conditions without swimming. Motor activity was automatically analyzed daily before swimming. Saturation studies were carried out in the presence of 8 increasing [^{3}H]-Quinuclidinyl benzilate ([^{3}H]-QNB) concentrations (0.0625-10 nM) in 0.05M PBS. Regional distribution of cholinergic muscarinic receptors was analyzed by autoradiographic methods, using 1 nM [^{3}H]-QNB. Non-specific binding was determined in the presence of 1μM atropine.

In the FST group, both total horizontal activity and ambulatory movements exhibited a significant decrease (34% and 46% respectively) when the data from 1st and 15th days were compared. [^{3}H]-QNB binding parameters were examined in the caudate-putamen, cortex and hippocampus. The Kd and Bmax values were very similar for both groups in the three analyzed areas, suggesting that neither the affinity of the [^{3}H]-QNB nor the maximal number of receptors were affected by the FST. The distribution of [^{3}H]-QNB binding sites did not show significant differences between both groups in the 30 studied areas. To rule out any cholinergic involvement, further analysis of muscarinic receptor subtypes after FST would be of interest since an alteration of one receptor subtype could be masked by [^{3}H]-QNB binding.

DRUGS OF ABUSE: COCAINE V

765.1

REPEATED ADMINISTRATION OF COCAETHYLENE PRODUCES SENSITIZATION TO ITS LOCOMOTOR EFFECTS

E. P. M. Prinssen*, M. S. Kleven and W. Koek. Centre de Recherche Pierre Fabre, Castres, France.

Cocaethylene, a metabolite resulting from combined ethanol and cocaine consumption, is a dopamine reuptake blocker and psychostimulant. That its effects, although similar, may not be identical to those of cocaine is suggested by the finding that, in rats, cocaethylene did not induce sensitization following repeated administration, nor did it induce cross-sensitization to cocaine, while the reverse was true for cocaine (Elsworth et al., Drug Dev Res 30, 189, 1993). To compare further the behavioral effects of repeated administration of cocaethylene, male C57BL/6J mice (n=5/group) were injected with cocaethylene (0, 10, 20, or 40 mg/kg, i.p.) for 3 consecutive days. On the 4th day, cocaethylene (0, 2.5, 10, 20, or 40 mg/kg, i.p.) was administered to all pretreatment groups. During daily sessions, mice were removed from their individual home cages and placed in an observation cage where locomotion was recorded for 30 min before and after injection using an automated activity monitor. Repeated injection of cocaethylene (10, 20, and 40 mg/kg/day x 3 days) produced apparent shifts of the cocaethylene dose-response function to the left, relative to that obtained in chronic saline treated animals. Cocaethylene pretreatment also induced an increase in the maximal effect compared to saline pretreatment (maximal beam breaks of 446 $\pm$ 53 and 205 $\pm$ 45, mean $\pm$ SEM, cocaethylene vs saline, respectively). These results demonstrate that cocaethylene produces a dose-dependent sensitization to its locomotor effects and, in this respect, is similar to cocaine which also showed sensitization under the present conditions. Therefore, together with the reported substitution of cocaethylene for cocaine in drug discrimination, the finding that cocaethylene, like cocaine, induces sensitization to its locomotor effects, suggests that both drugs share effects not only after acute, but also after chronic administration.

765.2

DIFFERENCES IN OPERANT BEHAVIOR OF RATS RUNNING FOR COCAINE VS COCAETHYLENE.

M. A. Raven*, B. D. Necessary, D. A. Danluck and A. Ettenberg. Behavioral Pharmacology Lab, Department of Psychology, University of California, Santa Barbara, CA 93106

The effects of cocaethylene, a psychoactive metabolite of cocaine produced in the presence of ethanol, were compared to cocaine using an operant runway self-administration paradigm. Rats were trained to traverse a straight-arm alleyway one trial per day for a single iv injection of cocaine or cocaethylene (0.5, 0.75, 1.0 or 2.0 mg/kg/inj). Infrared sensors located throughout the length of the runway were used to record the latency to initiate running (start latency), the time to enter the goal box (run time) as well as the intra-alley behavior of the animals. Although start times remained stable and fast, both drug groups demonstrated some ambivalence (approach-avoidance behavior) about entering the goal box. The incidence of this behavior, however, tended to increase with dose for cocaine and decrease with dose for cocaethylene. It is hypothesized that the strength of the positive relative to negative drug effects may be greater for cocaethylene than for cocaine, especially at higher doses.

765.3

COCAETHYLENE EXPOSURE DURING THE BRAIN GROWTH SPURT INDUCES BRAIN WEIGHT DEFICITS. W.-J.A. Chen*, R.L. Hernandez and J.R. West. Human Anatomy & Medical Neurobiology, Texas A&M University Health Science Center, College Station, TX 77843-1114

The increased prevalence of alcohol and cocaine abuse among women of reproductive age has received considerable attention since the concurrent use of these two substances results in the exclusive formation of a pharmacological active substance, cocaethylene (CE). Presently, it is unclear whether and, under what conditions, fetal/neonatal hepatic microsomal carboxylesterase activity is capable of transesterifying cocaine to CE in the presence of alcohol. Therefore, in this study we examined the direct action of CE, rather than the co-administration of alcohol and cocaine, on brain growth restrictions (microencephaly) during the major portion of the brain growth spurt. Three groups of artificially reared pups were given one injection of either 0, 10 or 20 mg CE (s.c.) daily, from postnatal days (PDs) 4 through 9. One group of normally reared pups (suckle control; SC) was included. All pups were perfused on PD 10 and the brains were removed and dissected into forebrain, cerebellum and brainstem. The analyses indicated that daily administration of either 10 or 20 mg CE produced a statistically significant restriction on brain growth in all three brain regions compared with the 0 mg CE group. However, no difference in brain weight deficits was found between two CE groups. Separate analyses between 0 mg CE and SC groups revealed no difference in brain measures. These results suggest that cocaethylene is teratogenic to the developing fetus and the concurrent use of alcohol and cocaine during pregnancy may be more harmful due to the unique formation of cocaethylene in addition to the intrinsic teratogenic effect of cocaine and alcohol individually. Supported by NIH grant DA07364.

765.4

PARTIAL DOPAMINE AGONISTS REDUCE ETHANOL INTAKE IN THE RAT L. Pulvirenti[1,2]*, C. Balducci[3], G. Bono[3], P. Richelmi[3] and G.F. Koob[1] [1]Dept. of Neuropharmacol., Scripps Res. Inst., La Jolla, Calif., [2]"Mondino-Tor Vergata" Ctr for Exp Neurobiol, Un. of Rome "Tor Vergata", Rome, [3]"C. Mondino" Fndtn., Pavia, Italy.

Ethanol is known to activate brain dopamine neurotransmission and the acute reinforcing properties of ethanol appear to be modulated by administration of drugs acting at the dopamine receptor site. Partial dopamine agonists are a recently characterized group of compounds which bind to dopamine receptors with high affinity and low intrinsic activity. These drugs act as functional antagonists in conditions of high dopamine tone, while they show an agonistic profile in conditions of dopamine depletion (e.g. denervation). The aim of the present study was to evaluate the effects of acute and chronic pretreatment with terguride and SDZ 208-911, two prototype partial dopamine agonists, in non-fluid deprived rats trained to drink alcohol (10 v:v) in a free-choice procedure. Acute treatment with both SDZ 208-911 (0.025-1.6 mg/kg) and terguride (0.025-4.0 mg/kg) dose-dependently reduced the reinforcing properties of ethanol as measured by lower intake. Similarly, chronic (10-day) treatment with both SDZ 208-911 or terguride (0.2 mg/kg) induced a significant reduction of ethanol intake. In no case was water intake affected thus ruling out possible nonspecific effects of these drugs on motor performance of consummatory behavior. Therefore, the unique pharmacological profile of partial dopamine agonists supports the hypothesis that these drugs may represent a novel potential therapeutic strategy for normalizing dopamine tone during the various phases of the natural history of drug dependence including alcoholism.

765.5

Effects of chronic alcohol and cocaine administration on brain gene expression. **German Torres* and Judith Pielecha. Behavioral Neuroscience Program. Department of Psychology. State University of New York at Buffalo. Buffalo, New York 14260.**

The concomitant use of alcohol and cocaine is the most common abuse pattern found in the addictive population. We have recently shown that this combination produces an antagonistic effect at the gene level, with alcohol blocking the transcription of *c-fos* by cocaine in brain neurons (G Torres, Synapse 18, October 1994). To explore this effect further, we investigated the long-term effects of combined alcohol and cocaine exposure on *c-fos* in discrete areas of the rat brain. Male rats were given a liquid diet containing alcohol (8.7%) for 15 days. Each night, two hr after the onset of darkness, the same rats were injected (ip) with cocaine (20 mg/kg). In addition, we determined the effects of cocaethylene (a psychoactive metabolite of cocaine produced by a liver carboxylesterase when alcohol is administered in conjunction with cocaine) on gene expression in order to compare its relative effects to those of alcohol and cocaine. Administration of alcohol and cocaine produced significant levels of plasma cocaethylene as detected by capillary gas chromatography and mass spectrometry. Further, chronic alcohol exposure markedly altered the induction of gene expression by cocaine in caudate putamen perikarya. The systemic injection of cocaethylene (20 mg/kg) also produced changes in *c-fos* in various regions of the rat brain including the caudate putamen, nucleus accumbens and cerebellum. Alcohol alone, in contrast, had no effect on gene transcription. Taken together, our data suggest that alcohol exerts a powerful inhibitory effect on cocaine actions, and that cocaethylene alone produces genomic effects similar to those seen after cocaine exposure.

This research was supported in part by a Term Faculty Development Award and a Research Foundation Award of the State University of New York to GT.

765.6

HUMAN COCAINE SELF-ADMINISTRATION: USE OF AN ALTERNATIVE REINFORCEMENT PROCEDURE TO EXAMINE POLYDRUG EFFECTS. John M. Roll*, Stephen T. Higgins, and Angela J. Desranleau. Dept. of Psychiatry, Human Behavioral Pharmacology Laboratory, University of Vermont, Burlington, VT 05401-1419.

A choice pardigm in which volunteers select from cocaine, placebo, alternative reinforcers (money) or elect not to respond will be described (See Higgins, et al., 1994 Life Sciences). This sensitive procedure demonstrates that cocaine self-administration varies as an orderly function of the magnitude of an alternative reinforcer (i.e., money). Said another way, as the magnitude of alternative reinforcer increases, cocaine self-administration decreases. This relationship provides a sensitive baseline to examine whether the effects of pretreatment with other drugs alters the behavioral control exerted by cocaine. Such an assessment is important because cocaine is frequently used in combination with other drugs of dependence. To date we have assessed the effects of ethanol pretreatment and have begun to assess the effects of caffeine pretreatment. Results indicate that both drugs alter preference for cocaine self-administration.

765.7

Characterization of a Novel Cocaine and Amphetamine Regulated Transcript (CART) P. Couceyro*†, M. Shoaib^, M. McCoy†, S. Goldberg^, M.J. Kuhar† Intramural Research Program, Neuroscience Branch†, Preclinical Pharmacology Lab^, Natl. Inst. Drug Abuse, NIH, Baltimore, MD 21224

Psychostimulant drugs such as cocaine and amphetamine can alter patterns of gene expression within neuroanatomical sites known to mediate drug reinforcement. Using PCR differential display, we previously identified a novel transcript (CART) rapidly induced only within the striatum by acute cocaine and amphetamine administration. In these studies, transcriptional regulation of CART was first assessed in the cocaine self-administration model. In limited daily access, Sprague-Dawley rats were trained to self-administer cocaine (0.66 mg/kg/infusion, i.v.) under a fixed ratio 3 schedule of reinforcement. Once behavior was maintained and response rates were stable for 5 consecutive days (less than 10% variability), animals were sacrificed 1 hr. after the last session. In 4 of 6 groups of animals, there was approximately a 50% reduction in CART mRNA levels in the nucleus accumbens (determined by Northern blot analysis) of rats self-administering cocaine compared with their saline-yoked controls. These data suggest a possible role for CART gene products in mediating the reinforcing properties of cocaine. Secondly, we now have evidence that passive acute cocaine and amphetamine administration increase CART mRNA levels within the nucleus accumbens and not in the caudate putamen.. The induction of CART by acute cocaine and amphetamine administration has been inconsistent, however. Future work will address this issue. Third, stress is known to alter the behavioral effects of drugs of abuse in animal models. We have begun to assess the effects of acute stress on CART expression. Within the nucleus accumbens, CART expression was decreased 1 hr. after either an i.p. saline injection, a 10 min. cold water swim and after 10 min. restraint. However, within the hypothalamus, CART mRNA levels were elevated by an i.p. saline injection, decreased after the cold water swim and unchanged by restraint. These studies strengthen the notion that CART may be involved in mediating the rewarding affects of cocaine and also indicate a role in response to stress.

765.8

DIFFERENTIAL EXPRESSION OF C-FOS FOLLOWING EXPOSURE TO A COCAINE- OR d-AMPHETAMINE-PAIRED ENVIRONMENT. **M.A. Klitenick*, C.-S. Tham and H.C. Fibiger. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1Z3**

The attribution of incentive salience to a drug-paired environment is of considerable clinical interest as drug craving can result from the presentation of stimuli previously associated with the effects of cocaine or d-amphetamine. In the present study regional expression of *c-fos* was examined immunohistochemically in rats exposed to an environment previously paired with either cocaine (10 mg/kg, i.p. X 10 days) or d-amphetamine (1.5 mg/kg, i.p. X 10 days). In addition to the conditioned motor-stimulant response elicited in animals exposed to the cocaine- or d-amphetamine-paired environment, *c-fos* expression was significantly increased in the cingulate and piriform cortices, claustrum, dorsomedial striatum, lateral septum, paraventricular nucleus of the thalamus and amygdala in those animals in which cocaine was previously paired with the environment. In addition, *c-fos* expression was significantly increased in the piriform cortex, dentate gryus and amygdala only in animals that were placed in an environment previously paired with d-amphetamine. These results indicate that the pattern of neuronal activation elicited by conditioned stimuli previously paired with d-amphetamine differs somewhat from that produced by a cocaine-paired environment. In contrast, acute administration of cocaine and d-amphetamine produce similar patterns of *c-fos* expression. Interestingly, although the nucleus accumbens is necessary for the unconditioned locomotor effects of cocaine and d-amphetamine, *c-fos* expression in this structure was not significantly altered by the contextual conditioned stimuli associated with either drug, thus indicating that different circuits may be involved in the conditioned and unconditioned effects of cocaine and d-amphetamine.

765.9

PLASMA CONCENTRATIONS OF COCAINE AND BENZOYLECGONINE FOLLOWING INTRAVENOUS COCAINE ADMINISTRATION. A.E. McCrea*, A.F. Lehner, R.M. Booze and C.F. Mactutus, Univ. of Kentucky College of Medicine, Dept. of Pharmacology, College of Pharmacy, THRI and Graduate Center for Toxicology, Lexington, KY 40536.

Following either s.c. or i.p. injection, plasma cocaine concentration is characterized by slow first order kinetics and prolonged elevation. The present study examined the plasma concentrations of cocaine and benzoylecgonine (BE) following a single i.v. injection of 0.5 mg/kg, 1.0 mg/kg or 3.0 mg/kg cocaine. Male Sprague-Dawley rats (N=32) were fitted with a subcutaneous vascular access port (Mactutus et al., 1994) and allowed to recover for at least 3 days. Plasma samples (200 µl) were obtained at 8 time points (30, 60, 90, 120 seconds and 5, 10, 20, 30 minutes) following injection and analyzed by single ion monitoring using GC/MS. Derivatization of BE to the trimethylsilyl ester (TMS) was performed prior to analysis and the 240/243 m/e ion pair ratio was interpolated to a 9 concentration (0-10,000 ng/ml) standard curve. Identical SIM procedures were established utilizing the cocaine 182, 198, 272 and 303 m/e ion pairs and the plasma concentration of cocaine was determined by the interpolation to the 185 m/e peak of cocaine-d_3. Peak plasma concentrations of cocaine occurred at 30 seconds in a dose-dependent manner (3.0>1.0>0.5 mg/kg). In both 0.5 and 1.0 mg/kg cocaine groups, plasma concentrations decreased to <200 ng/ml by 10 minutes, yet the 3.0 mg/kg group remained elevated (>500 ng/ml) for longer than 20 minutes. Plasma concentrations of BE reached a plateau in the 0.5 mg/kg (80-90 ng/ml) and the 1.0 mg/kg group (160-170 ng/ml) within 10-20 minutes whereas the concentration of BE in the 3.0 mg/kg group was increasing at 30 minutes (>500 ng/ml). The close parallel of the present data to human cocaine kinetics (Evans et al., 1994) indicates the s.c. vascular access port model of i.v. cocaine administration offers a viable alternative to i.p. or s.c. routes for the study of behavioral modifications and neuropharmacology following cocaine administration. (Supported by DA06638, DA09160, & ES06259)

765.10

DIFFERENTIAL ACTIONS OF COCAINE AND AMPHETAMINE ON SOMATOSENSORY CORTICAL NEURONS. F.M. Sessler*, T.N. Felder, J. Zhai, F.-C. Hsu, R.C.-S. Lin, B.D. Waterhouse, and J. Lehmann. Depts. Of Anat. & Neurobiol., and Neurosurg., Med. Col. Of Pennsylvania and Hahnemann Univ., Philadelphia, PA 19102

Cocaine and amphetamine have both psychostimulant properties. Some of their effects have been related to an enhancement of monoaminergic transmission by blockade of reuptake and/or direct release of monoamines. The question whether these two compounds produce similar or differential actions on sensory cortical circuits and pyramidal neurons membrane was investigated here. Experiments were conducted using an in vitro brain slice preparation from rat somatosensory cortex. Intracellular recordings of cortical neurons were made before, during and after bath application of cocaine or amphetamine (0.1-100µM). EPSPs were evoked by electrical stimulation of the underlying white matter. At low concentration (0.1-1µM), both cocaine an amphetamine increased spike firing probability and EPSP amplitude of cortical neurons. At higher concentrations (10-100µM), bath application of cocaine was predominantly inhibitory whereas amphetamine produced both excitatory and inhibitory effects over these same parameters depending on the cell tested. These results suggest that different monoamines system may be involved with higher concentrations of amphetamine. Also, cocaine-induced local anesthetic action may interact and cancel psychostimulant effects of the drug on cortical circuits. Furthermore, identification of vulnerable cell types may be a necessary step in predicting the impact of these drugs on somatosensory cortical circuits. (Supported by NIDA DA08405 to FMS and DA05117 to BDW).

765.11

PRECLINICAL ASSESSMENT OF THE POTENTIAL USE OF METHYLPHENIDATE AS A TREATMENT FOR COCAINE ABUSE C. McNamara* and S. Schenk, Texas A&M University, Dept. Psychology, College Station, TX 77843

Three different self-administration paradigms were used in an assessment of the potential use of methylphenidate as a substitution pharmacotherapy for the treatment of cocaine abuse. In the first, rats were trained to self-administer cocaine and then were treated with methylphenidate (0.0 or 20.0 mg/kg) immediately prior to daily 2 hr sessions. Methylphenidate reduced cocaine-reinforced responding across a wide range of cocaine doses (0.03-0.50 mg/kg/infusion) to control levels suggesting that the reinforcing effects of cocaine were completely blocked by pretreatment with methylphenidate. However, methylphenidate was reliably self-administered and, in a model of drug seeking behavior, it dose-dependently reinstated extinguished cocaine-taking. Taken together, these data suggest that methylphenidate can effectively block cocaine self-administration. However, the drug has potential for abuse, as indicated by its self-administration and may serve as a cue to reinstate drug-seeking behaviors.

Supported by DA06825.

765.12

rGβ: A NOVEL RAT G PROTEIN β SUBUNIT WHOSE EXPRESSION ALTERS PROCESS FORMATION IN SHY5Y CELLS **@#G.R.Uhl,* X.B.Wang@.. Mol. Neurobiol. Br.@, IRP, NIDA, NIH; Dept. #Neurol. & Neurosci., JHUSM, Balto., MD 21224.**

βγ dimers resulting from G protein activation may transduce cellular signals from a variety of extracellular stimuli, including abused drugs. During searches for drug-regulated genes, we identified a partial cDNA clone that demonstrated striking regulation by psychostimulants and morphine as 80% homologous to 3' sequences of the human G protein β_1 subunit. Screening produced a 2.9 kb cDNA that contained an open reading frame of 1020 bp sequence and a predicted amino acid sequence 98% identical to the hGβ_1 sequence but only 72% or less identical to previously-reported rat Gβ sequences. This apparent rat G protein β_1 subunit was expressed most strongly in brain, with different levels noted in different brain regions. Striking morphological changes were observed when mRNA sense and antisense rGβ_1 constructions were expressed in SY5Y neuroblastoma cells. Transfection with mRNA sense rGβ_1 resulted in slow growth and loss of neuronal-like processes from these cells. In contrast, cells transfected with the antisense construction displayed more vigorously extended neural process and expanded growth cones. If these *in vitro* findings are reproduced in neurons, rGβ_1 may play a significant role in drug-induced synaptic plasticities.

765.13

GENES REGULATED BY PSYCHOSTIMULANTS AND MORPHINE IN BRAIN *X.B.Wang*@, H. Ujike@, J.T.You@ and G.R.Uhl#@*. @Mol. Neurobiol. Br., IRP, NIDA, NIH; #Dept. Neurol. & Neurosci., JHUSM, Balto., MD 21224

Although molecular mechanisms establishing or maintaining long-term behavioral changes induced by abused drugs are largely unknown, drug-induced alterations in neuronal gene expression could play significant roles in these manifestations of neuronal plasticity. We have used subtracted differential display PCR to identify an initial set of 20 genes characterized by altered expression following psychostimulants or morphine. Differentially-expressed cDNAs hybridize to mRNAs ranging in size from 0.5 to 12kb. Six display brain-specific expression. Sequence analyses reveals several with homologies to neurotransmitter receptor, intracellular signal transducing factor, and stress responsive enzyme gene families. These and other drug-regulated cDNAs encode candidate genes for involvement in long term CNS changes induced by abused drugs.

765.14

FACILITATION OF BRAIN STIMULATION REWARD BY MILDLY PSYCHOACTIVE SUBSTANCES: A QUANTITATIVE COMPARISON WITH PROTOTYPIC ADDICTIVE DRUGS. M.A. Bozarth*, C.M. Pudiak, & R. KuoLee. *Department of Psychology, University at Buffalo, Buffalo, NY 14260-4110.*

Considerable evidence suggests that a compound's effect on brain stimulation reward (BSR) provides a useful assessment of its potential addiction liability. Drugs that enhance BSR are generally highly addictive, and drugs that are not addictive usually fail to enhance BSR. Most investigators consider this a qualitative indicator: BSR facilitation suggests the drug has addictive properties. However, earlier work (M.A. Bozarth, *Intracranial self-stimulation as an index of opioid addiction liability* . . . . Unpublished M.A. thesis: RPI, 1978) suggested that quantitative aspects of facilitation may be important in distinguishing compounds with high and low addiction liabilities. The present study examined the facilitatory effects of three compounds: the prototypic addictive drug cocaine, the nonaddictive decongestant pseudoephedrine, and nicotine.

Male, Long-Evan rats with lateral hypothalamic stimulating electrodes were tested using a threshold tracking procedure. Daily 30-min test sessions determined the minimum stimulation frequency necessary to maintain responding at ≥30 presses/min. Cocaine hydrochloride (1 to 30 mg/kg, i.p.), pseudoephedrine hydrochloride (3 to 100 mg/kg, i.p.), or nicotine bitartrate (0.125 to 1 mg/kg, s.c.) were injected immediately before threshold tracking. All drug injections were separated by a minimum of 72 hrs. Peak threshold-lowering effects were determined using 180-min test sessions.

Cocaine produced robust threshold lowering. Pseudoephedrine also lowered BSR thresholds, but this effect was much less than that seen with cocaine (i.e., <25% vs. >50% threshold reduction, respectively). Nicotine produced facilitation quantitatively similar to that seen with pseudoephedrine and distinct from the effect seen following cocaine. These data suggest that quantitative aspects of facilitation must be considered to distinguish compounds with a high addiction liability (e.g., cocaine) from substances with a low addiction liability (e.g., pseudoephedrine). These data further suggest that nicotine's profile in this animal model is that of a nonaddictive substance.

765.15

THE EFFECTS OF MEDIAL PREFRONTAL CORTEX LESIONS ON COCAINE SELF-ADMINISTRATION AND CONDITIONED COCAINE-SEEKING BEHAVIOUR IN RATS. R. Weissenborn*, T.W. Robbins and B.J. Everitt
Department of Experimental Psychology, University of Cambridge, Cambridge CB2 3EB, UK

The medial prefrontal cortex (mPFC) may have an important function in mediating the reinforcing effects of psychostimulant drugs - most likely through its role within corticostriatopallidal feedback loops and specifically through its afferent connections with the ventral striatum. The present series of experiments was designed to examine in detail the involvement of the mPFC in different aspects of conditioned and unconditioned cocaine-seeking behaviour in rats. Quinolinic acid-induced excitotoxic lesions of the mPFC resulted in significantly shorter inter-injection intervals and higher response rates during the acquisition and maintenance of i.v. cocaine self-administration, compared to sham-lesioned controls ($p<0.05$). This lesion effect was specific to cocaine-reinforced responding, since both groups extinguished responding on a control lever at the same rate. Within- and between-session dose-effect curves to cocaine were also determined. Medial PFC lesions produced significant increases in response rates both on the ascending limb and at the top of the descending limb of the inverted U-shaped dose-effect curve, but no shift to the left of the entire function was observed. When responding resulted in saline infusions only, lesioned rats continued to discriminate between active and inactive levers, while control rats responded on both levers at equal rates. Together, these observations support the hypothesis that excitotoxic lesions of the mPFC may enhance the propensity for perseverative responding rather than alter cocaine's ability to maintain operant responding. Preliminary data further suggest that mPFC lesions may impair responding maintained by conditioned reinforcers under second-order schedules of cocaine reinforcement, indicating a deficit in the extent to which cocaine-seeking behaviour is controlled by drug-associated cues. This work was supported by MRC Grant G9407194N.

765.16

OPENFIELD AND PASSIVE AVOIDANCE BEHAVIOR OF YOUNG ADULT RATS PRENATALLY EXPOSED TO COCAINE, ETHANOL OR BOTH DRUGS OF ABUSE. K. Foley* and C. Ulibarri, Dept of VCAPP, Washington State University, Pullman WA 99164-6520.

Drugs of abuse such as cocaine and alcohol are known to cause behavioral deficits. When cocaine and alcohol are abused simultaneously, the transesterification product, cocaethylene, is formed. Cocaethylene is more potent than cocaine in some systems, and is known to cross the placenta barrier. Few studies have characterized the effects of alcohol and cocaine used concurrently in an animal model. In this study, the effects of prenatal exposure to cocaine, alcohol, or both drugs of abuse on activity in an openfield as well as memory retention in a passive avoidance paradigm were studied. Timed-pregnant Sprague-Dawley dams were treated on gestational days 11-21 with one of the following: 1) free-fed 2) nutritionally matched to drug-treated dams (pair-fed) 3) cocaine (20 mg/kg) 4) low dose of alcohol (EtOH-lo; 30% of calories as alcohol) 5) high dose of alcohol (EtOH-hi; 60% of calories as alcohol), 6) EtOH-lo and cocaine, 7) EtOH-hi and cocaine. At birth, pups were fostered to surrogate dams to avoid possible maternal postnatal effects of the drugs. As adults, rats were tested in four weekly openfield behavior tests followed by training on a standard dark-light passive avoidance paradigm.

In openfield behavior tests males from the EtOH treatment groups demonstrated significantly reduced margin time as compared to pair-fed controls. These males also had a significantly increased level of horizontal activity and a significantly decreased level of vertical activity. In a passive avoidance paradigm, males showed a significantly greater latency to enter after shock conditioning than females. Alcohol treatment significantly increased the latency to enter in the passive avoidance paradigm. Supported by funds provided for medical and biological research by the State of Washington Initiative Measure No. 171 to CU.

DRUGS OF ABUSE: COCAINE VI

766.1

THE RELATIONSHIP BETWEEN COCAINE SELF-ADMINISTRATION AND SACCHARIN PREFERENCE. B.A. Gosnell*, D.D. Krahn and J.M. Yracheta.
Department of Psychiatry, University of Wisconsin-Madison, Madison, WI 53792.

Previous research suggests a positive relationship between saccharin preference and the self-administration of ethanol (orally) and morphine (i.v.) in rats. This experiment was performed to determine whether a similar relationship exists between saccharin preference and cocaine self-administration. Male rats (n=32) were given *ad lib* access to saccharin and water for 4 days. Total daily fluid intake over days 3-4 was then expressed as a percentage of intake when only water was available. Rats varied in the degree to which saccharin increased total intake, with values ranging from 99 - 237% of baseline water intake. Jugular catheters were then implanted in all rats. After recovery, cocaine self-administration was measured in an overnight session (18 hr, FR 1 schedule, 0.4 mg/kg/infusion with a cutoff of 80 mg/kg). Some rats (n=8) across the entire range of saccharin preferences obtained the maximum number of infusions. Interestingly, of the remaining 24 rats, those with intermediate saccharin preferences self-administered more cocaine than rats with low or high preferences; in these rats, the difference between low and intermediate rats was significant ($p<0.05$). Following the overnight session, all rats were tested in daily 1 hr sessions at doses of 0.125 - 1.0 mg/kg/infusion and with reinforcement schedules of FR1 - FR6. A significant relationship between saccharin preference and cocaine self-administration was observed only under conditions of a FR 6 schedule combined with a low dose (0.125 mg/kg/infusion). Under these conditions, rats from the middle third of the distribution of saccharin preferences self-administered more cocaine than rats in the upper or lower thirds. This "inverted U"-shaped relationship between saccharin preference and cocaine self-administration differs from that observed with ethanol and morphine. These results may be related to the observation by Glick et al. (Brain Res. 653:148-154, 1994) of a curvilinear relationship between baseline levels of dopamine in the nucleus accumbens and cocaine self-administration.
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766.2

PREEXPOSURE TO COCAINE ATTENUATES COCAINE-INDUCED TASTE AVERSIONS. H.F. Diamond* and A.L. Riley.
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Animals exposed to one of a number of compounds prior to taste aversion conditioning with those compounds display attenuated aversions relative to nonpreexposed subjects. In the present experiment, the effect of exposure to cocaine on cocaine-induced aversions was assessed. Cocaine was examined within this preparation because in other designs chronic exposure to cocaine potentiates (or sensitizes) subsequent responsivity to cocaine. In the present design, rats were administered cocaine (32 mg/kg, SC) or vehicle every fourth day for a total of five drug exposures. Animals were then given a novel saccharin solution to drink and immediately injected with cocaine (32 mg/kg, SC) or the distilled water vehicle. Animals preexposed to vehicle and injected with cocaine during conditioning rapidly acquired a taste aversion to cocaine. Animals preexposed to cocaine and injected with cocaine during conditioning failed to acquire a taste aversion, drinking at levels comparable to controls (i.e., subjects preexposed to cocaine but injected with vehicle and subjects preexposed to the vehicle and injected with vehicle). Similar to other drugs within this preparation (both abused and nonabused), cocaine preexposure weakens (and does not potentiate) subsequent cocaine-induced taste aversions.

766.3

THE INTERACTION OF ALCOHOL AND COCAINE IN TASTE AVERSION CONDITIONING. B.-F. X. Sobel*, S. A. Etkind, W. E. Fantegrossi and A. L. Riley. Psychopharmacology Laboratory, Department of Psychology, The American University, Washington, DC 20016.

In prior work (Sobel & Riley, Coll. Prob. Drug Dep., 1994), we have demonstrated that doses of alcohol and cocaine without effect on schedule-controlled behavior markedly suppressed responding when given in combination, an effect consistent with work from a number of other behavioral and physiological preparations in which alcohol has been reported to potentiate the effects of cocaine. In the present experiments, the interaction of these compounds was assessed within the taste aversion preparation, a behavioral index of toxicity. In Experiment 1, different groups of rats were given 20-min access to a novel saccharin solution followed immediately by an injection of either alcohol (0.56 g/kg, ip), cocaine (25 mg/kg, sc), the distilled water vehicles or the alcohol/cocaine combination. Although neither drug alone (nor the vehicle injections) induced a taste aversion, animals injected with the combination markedly decreased saccharin consumption, acquiring an aversion to saccharin after only two saccharin-alcohol/cocaine pairings. Interestingly, alcohol did not potentiate cocaine-induced aversions when cocaine was given ip, although the same combination dramatically affected schedule-controlled behaviors (Experiment 2). These data indicate that the interaction of alcohol and cocaine (when administered subcutaneously) extends to indices of toxicity.

766.4

COCAINE HAS DIRECT EFFECTS ON THE CORTICOTROPHS (AtT-20) L.M. Konopka*, R.J. Wong, S.M. Delisi and J.W. Crayton. Biological Psychiatry Section,
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Cocaine abuse is a major public health problem. Cocaine addicts frequently present with psychiatric as well as neuroendocrine abnormalities. Cocaine's influence on the neuroendocrine system has been investigated. Primary research efforts have been directed towards elucidating the hypothalamic mechanisms of cocaine's action. To date however, no studies addressed direct effects of cocaine on the pituitary cells. We have selected a well-characterized model of anterior pituitary corticotrophs, the AtT-20/D16v cell line, to elucidate the direct effects of cocaine on a single component of the neuroendocrine system. Whole cell recordings utilizing current and voltage clamp techniques was employed. In addition, intracellular calcium was measured by means of Fura-2 imaging techniques. Images of both cell populations and single cell recordings were acquired. Application of cocaine (300 nM - 3 micM) resulted in inhibition of spontaneous firing. This inhibition was associated with significantly increased outward and to lesser extant inward currents. Results from the population based calcium recordings indicated cocaine induced increase in the resting calcium levels. Single cell recordings support the population studies. The results of this study point to a complex mode of cocaine's action on the pituitary corticotrophs and its possible role in the modulation of the excitation secretion processes.

766.5

THE EFFECTS OF REPEATED COCAINE ADMINISTRATION ON PERGOLIDE-INDUCED ENDOCRINE RESPONSES IN HUMAN DRUG USERS. M.H.Baumann*, K.M.Becketts, T.M.Gendron, J.E.Henningfield, D.A.Gorelick and R.B.Rothman. Clinical Pharmacology Lab., IRP, NIDA, NIH Baltimore, MD 21224.

Preclinical evidence indicates that chronic cocaine exposure alters dopamine (DA) neurotransmission. In the present study, we examined the effects of repeated intranasal cocaine administration on DA function in human subjects in an in-patient research study. Male polydrug abusers (N=11) participated in 2 daily self-administration sessions (AM & PM) for 5 consecutive days. Subjects snorted both placebo and cocaine (96 mg) each day, with cocaine being presented in a randomized, double-blind fashion at 1 of the daily sessions. Neuroendocrine challenge tests were performed using the DA receptor agonist pergolide (0.1 mg, p.o.) 5 days before and 3 days after the repeated cocaine dosing regimen. Repeated blood samples were drawn at 30 min intervals from 1 hr before until 6 hr after pergolide challenge. Plasma prolactin (PRL) and growth hormone (GH) concentrations were determined by RIA. Pergolide decreased circulating PRL and increased GH. No significant differences in responsiveness to pergolide were found between the pre- and post-cocaine condition. Our data suggest that repeated doses of intranasal cocaine do not modify DA receptor sensitivity in human drug users.

766.6

INFLUENCE OF CORTICOSTERONE ON THE PSYCHOMOTOR EFFECTS OF COCAINE: A DOSE-RESPONSE STUDY. M. Marinelli, F. Rougé-Pont, M. Le Moal and P.V. Piazza*. INSERM U. 259, Université de Bordeaux II. 33077 Bordeaux Cedex, France.

Adrenalectomy (ADX) is known to reduce the behavioral effects of cocaine whereas replacement of corticosterone in the range of basal diurnal levels reverses these effects. This study further investigated the relationship between corticosterone (CORT) and sensitivity to psychostimulants. For this purpose, in a first experiment we studied the effects of ADX on the locomotor response to different doses of cocaine (0, 3.25, 7.5, 15, 30, 60 mg/kg). Locomotor activity increased with the increase in drug concentration reaching the maximal locomotor activation at the dose of 30 mg/kg in all animals, however the maximal effect of the drug was reduced by 60% by removal of circulating of CORT. Thus, ADX rats did not differ from controls either for the response to a saline injection or for the psychomotor activation induced by the lower doses of cocaine (3.125 and 7.5 mg/kg), but at all of the higher doses ADX rats showed a lower locomotor response than sham rats. In a second experiment we investigated the dose-response effects of replacing circulating CORT in ADX rats on the locomotor response to cocaine (20 mg/kg). In this study ADX animals were implanted with subcutaneous pellets containing different concentrations of the hormone (0, 3.125, 12.5 or 50 mg) so as to progressively increase circulating CORT to levels observed in diurnal conditions. Again, the absence of CORT reduced the locomotor response to cocaine and there was a progressive increase in drug effect with increasing doses of CORT. The effects of ADX were completely abolished by the higher dose of CORT which gave diurnal levels of the hormone. The differences observed in response to cocaine cannot be explained by a difference in drug availability since neither ADX nor CORT replacement had any effects on brain levels of cocaine. These results indicate that stimulant effects of cocaine depend on CORT concentrations and that this hormone facilitates the psychomotor properties of this drug. This study suggests that individual differences observed in sensitivity to drugs of abuse may depend on circulating CORT levels and may open new therapeutic strategies for drug addiction.

766.7

CHRONIC COCAINE TREATMENT DECREASES OXYTOCIN LEVELS IN THE AMYGDALA AND INCREASES MATERNAL AGGRESSION IN SPRAGUE DAWLEY RATS. **J.M.Johns*, B.M.Faggin, L.R.Noonan, L.Li, L.I.Zimmerman and C.A. Pedersen. Dept. of Psychiatry, Univ. of North Carolina, Chapel Hill, N.C. 27599.**

Gravid Sprague-Dawley rats (250-275g) received one of three treatments throughout gestation; s.c. injections b.i.d. of saline (Sal) or 15 mg/kg of cocaine HCL or 1.5 mg/kg amfonelic acid (AFA) once daily. Females were tested on postpartum day 6 for maternal aggression towards a male intruder during a 10 min. period. Cocaine treated dams threatened intruders more than AFA dams (p<.04) and attacked intruders more than AFA (p<.05) or Sal (p<.04) treated dams. Dams were sacrificed for oxytocin RIA's using the whole ventral tegmental area and amygdala. Mean picograms per area and per mg protein were compared for the groups. Chronic cocaine treatment significantly reduced oxytocin levels in the amygdala as compared to Sal-treated (p<.05) and AFA-treated dams (p<.01). **(Supported by NIH grant DA08456 and a UNC Medical Faculty Grant).**

766.8

NEUROENDOCRINE AND BEHAVIORAL RESPONSES TO COCAINE iN FISCHER AND LEWIS RATS M. Renee Simar* and Nick E. Goeders. Dept. of Pharmacology and Therapeutics. Louisiana State University Medical Center, Shreveport, LA 71132.

Differences in the vulnerability to self-administer drugs of abuse have been observed in laboratory rats. Reports suggest that differences in adrenocortical activity may be a factor in the variability to acquire drug self-administration. These studies compared the cocaine-induced neuroendocrine and behavioral responses in strains of rats previously shown to self-administer drugs differently. Lewis (LEW) and Fischer (F344) rats were chosen for their pattern of self-administration as well as for differences in the responsiveness of the hypothalamic-pituitary-adrenocortical (HPA) axis to various stimuli. The acute neuroendocrine responses to cocaine were characterized by the effect of cocaine (1-60 mg/kg, i.p.) on hypothalamic corticotropin-releasing factor (CRF), corticosterone (CS), and adrenocorticotropin hormone (ACTH). Although both LEW and F344 rats displayed a decrease in brain CRF and an increase in plasma CS and ACTH, the percent response in LEW rats was greater than in F344 rats. This suggests that acute injections of cocaine can stimulate HPA axis activity, and that LEW rats, the drug-preferring strain compared to F344 rats, are more sensitive to the neuroendocrine activating effects of cocaine. Studies on the reinforcing effects of cocaine using an operant model of intravenous (i.v.) administration have also been performed. Rats were tested for acquisition of cocaine self-administration at increasing doses (0, .031, .062, .125, .250, .500, and 1.00 mg/kg/infusion). Our data indicate that LEW rats will self-administer i.v. cocaine more readily than F344 rats. Although basal plasma CS is higher in F344 rats, a greater CS response to i.v. cocaine was observed in the LEW strain. These studies suggest that neuroendocrine and behavioral responses to cocaine are markedly different in LEW and F344 rats , and these differences may be a factor in the variability in drug preference observed in these strains. This work was supported by USPHS Grant DA06013.

766.9

INCREASED COCAINE SELF-ADMINISTRATION AFTER SOCIAL STRESS. K.A. Miczek*, N. Hubbard and I. Cantuti-Castelvetri. Dept. Psychology, Tufts Univ., Medford MA 02155.

Cocaine and many kinds of stress lead to increased release of mesocorticolimbic dopamine. How specific is the activational effect of social stress to cocaine self-administration? We assessed the impact of social stress on behavior that was reinforced either by i.v. cocaine or by food, each at similar rates. Social stress was engendered by exposing the experimental rat solely to the threats of an aggressive resident rat behind a protective screen without potentially injurious attacks. Initially, we determined the characteristic inverted U-shaped concentration-response rate curve for cocaine self-administration (0.016-0.25 mg/infusion), maintained by an FR 10 schedule. Each cocaine dose was assessed for 3-5 days. In a second phase, animals were exposed to social stress for 60 min immediately before the daily session, and the cocaine concentration-response rate curve was re-determined. In a parallel group, the rate of food-reinforced behavior, maintained by a joint FR 10 FI 3 min schedule, was determined before, during, and after daily exposure to social stress. The rate of i.v. cocaine self-administration, but not of food-reinforced responding, was increased in rats that were socially stressed prior to the daily session. Other stressors like pair housing or exposure to a novel environment did not affect cocaine self administration. This increase was largely due to a high rate of responding immediately after the cocaine infusion pointing to activational effects of the self-administered cocaine in animals whose mesocorticolimbic dopamine is already released due to social stress.

766.10

SOCIAL STRESS MODULATES FOS EXPRESSION AFTER COCAINE AND MORPHINE TREATMENT IN MICE. E.M. Nikulina*, J.E. Marchand, K.A. Miczek and R. Kream. Department of Anesthesiology and Department of Psychology, Tufts University, Medford, MA 02155

Previous stress may change vulnerability to drugs of abuse, however neither the neural mechanisms underlying such effects nor the nature of the stress are well understood. The objective was to study whether social stress, either one week or immediately before administration of morphine or cocaine can influence c-fos expression in brain stem of mice. "Social stress" was defined as defeat in a brief confrontation with aggressive resident mouse and subsequent exposure to the resident's threats behind a protective screen. Mice were injected i.p. with morphine (7.5 mg/kg) or cocaine (40 mg/kg) and perfused 1 hour after injections. c-Fos immunoreactivity protein was investigated in periaqueductal gray (PAG) and locus coeruleus (LC). Social (defeat) stress in naive mice increased Fos-positive cells in PAG and LC in comparison to saline or untreated mice. Administration of morphine enhanced c-fos expression in PAG and LC. Social stress immediately before morphine injection appeared to have additive effects on c-fos expression in PAG and LC, whereas social stress one week before morphine challenge attenuated c-fos expression in PAG. Cocaine produced large c-fos expression 1 hour after injection in PAG and LC. A single defeat immediately before cocaine significantly attenuated c-fos expression in PAG and LC, whereas social stress 1 week before cocaine administration diminished c-fos expression in LC, resulting in ca. 50% lower count than after acute cocaine. These results suggest that development of stress-induced tolerance or sensitization to morphine or cocaine, respectively, is related with c-fos expression in brain stem.

766.11

VARIABLE HEMODYNAMIC RESPONSIVENESS TO BEHAVIORAL STRESS AND COCAINE IS RELATED TO DIFFERENTIAL CNS RESPONSIVITY. M.M. Knuepfer*, D. Hoang, P.J. Mueller, Q. Gan, L. Vickery and T.C. Westfall. Pharm. & Physiol. Sci., St. Louis Univ., St. Louis MO 63104.

We have noted highly variable hemodynamic responsiveness to cocaine and air jet stress in a population of rats (AJP, 265:H779-H782, 1993). In the present study, we examined the relationship between cardiovascular responsiveness to cocaine and to air jet with release of dopamine (DA) and norepinephrine (NE) in the nucleus accumbens (NA) and striatum (Str) as measured by HPLC. Conscious rats instrumented for determination of changes in cardiac output (CO) using pulsed Doppler velocitometry were tested for hemodynamic responsiveness to cocaine (5 mg/kg, *i.v.*) and air jet stress. Subsequently, rats were prepared with microdialysis probes in the NA and Str. On the following morning, rats were acclimated then physically restrained for 20 minutes while collecting dialysates. In the afternoon, cocaine was administered as before. As previously reported, stress or cocaine elicited pressor responses by increasing systemic vascular resistance only (vascular responders) or by increasing both CO and vascular resistance (mixed responders). The mixed responders had higher resting NE levels in Str. Restraint stress elicited increases in Str NE only in mixed responders whereas cocaine elevated Str DA levels more in vascular responders. These data suggest that differential hemodynamic responses to cocaine or stress are related to variable levels and release of catecholamines in the striatum. (Supported by DA05180).

766.12

DIFFERENTIAL EFFECTS OF ACUTE RESTRAINT STRESS ON THE BEHAVIORAL EFFECTS OF COCAINE. J.B. Acri and J.M. Witkin* Drug Development Group, Psychobiology Section, NIDA-DIR-ARC, NIH, Baltimore, MD 21224.

Acute laboratory stressors result in a number of neurochemical and behavioral effects. Stress has been reported to increase dopamine levels in brain regions associated with subjective and reinforcing effects of abused drugs, and has been reported to produce cross sensitization to psychomotor stimulants. The present experiments were undertaken to determine if acute restraint stress could modify the discriminative stimulus effects and the locomotor stimulatory effects of cocaine. Six rats trained to discriminate 10 mg/kg of cocaine were restrained for 15-20 minutes, and the dose-effect curve of cocaine was significantly shifted to the left, suggesting a potentiation of subjective effects. Subsequent re-establishment of the dose-effect curve indicated that the stress effect was transient, by a return to baseline values. A second series of experiments evaluated the effects of 15-20 minutes of restraint stress on the locomotor stimulant effects of cocaine in mice. Naive mice were either restrained for 15-20 minutes or remained in home cages prior to administration of saline or one of several doses of cocaine. Stress significantly decreased locomotor activity, measured as horizontal activity or number of movements, and significantly reduced cocaine-induced increases. When this experiment was repeated with mice that had been previously habituated to the locomotor arena, stress reduced locomotor activity in saline-treated, but not in cocaine-treated mice. These results suggest that stress effects on cocaine-mediated behaviors are complex, are dependent upon the behaviors being measured, and can be further modulated by behavioral history.

766.13

CHRONIC EXPOSURE TO CADMIUM ALTERS THE INITIATION OF BEHAVIORAL SENSITIZATION TO COCAINE. J.R. Nation*, C.L. Livermore, and G.R. Bratton. Texas A&M University, College Station, TX 77843

Cadmium selectively accrues in tobacco plants and poses a significant health hazard to heavy users of tobacco products. Recent findings indicate that risks may extend to include altered sensitivity to drugs that have substantial abuse liability. Continuing this line of research, the present investigation examined the effects of recurrent cadmium exposure on behavioral sensitization to cocaine. While monitoring motor activity, control and cadmium-treated adult male rats [60 days exposure to 100 ppm cadmium chloride via water supply], received single daily IP injections of 10 mg/kg cocaine HCL or saline for 14 successive days. On Day 15 all rats received saline injections, followed on Day 16-18 with incremental challenges of 10, 20, and 40 mg/kg cocaine. The findings showed that cadmium exposure retarded the initiation of sensitization. These data suggests that xenobiotic contamination may alter drug responsiveness and therein may influence patterns of drug selection and use.

766.14

NEUROBIOLOGICAL RELATIONSHIP BETWEEN VULNERABILITY TO DEPRESSION AND TO DRUG ABUSE: *IN VIVO* MICRODIALYSIS STUDIES. Marino Lepore* and Eliot L. Gardner. Department of Psychiatry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461-1602.

Clinical studies have shown a marked association between co-vulnerability to depression and to drug abuse. In agreement with theories that depression is reflected in a reduced capacity to experience pleasure, animal experiments demonstrate that manipulations that predispose towards depression also influence reward processes. Changes in neurotransmission in the mesolimbic dopamine (DA) system may underlie this effect. Stress has been shown to increase DA turnover in this system and mild /severe stress can induce abnormalities in DA transmission specific to the nucleus accumbens (NAcc). The following experiment examined the relationship between the co-vulnerability to depression and to drug abuse. The effect of cocaine on the development of learned helplessness (LH) was studied in animals genetically susceptible (Lewis-L)/non-susceptible (Fischer 344-F344) to drug abuse. Following exposure to LH, animals were implanted with dialysis probes in the NAcc, and the effect of cocaine challenge (10 mg/kg) on DA transmission was examined. Although L rats are more susceptible to drug abuse than F344, neither strain demonstrated any predisposition towards LH. When cocaine was administered prior to LH training, 40% of L and 70% of F344 rats became non-LH. Cocaine, therefore, had a protective effect on the development of LH, and was more pronounced in F344 rats. Following cocaine challenge, dialysate DA levels were heavily influenced by previous LH exposure. For controls, dialysate DA was higher in L rats than F344. Following LH training, dialysate DA levels did not increase significantly in F344 rats compared to controls. However, F344 rats that received cocaine during LH training (and who later became non-LH) showed a greater than 300% increase in dialysate DA levels. The effects of cocaine on dialysate DA in the L rat are currently under investigation. Thus, when cocaine is administered during LH training, the effect on dialysate DA levels in F344 rats is dramatic, and suggests that a potentially significant and long-lasting change in the mesolimbic DA system following cocaine+stress could reverse a genetic non-susceptibility to drug abuse.

766.15

EFFECT OF ADRENALECTOMY ON INITIATION AND EXPRESSION OF COCAINE- INDUCED SENSITIZATION AND STRESS- INDUCED CROSS SENSITIZATION. B. M. Prasad , C. Ulibarri, P. Duffy,*P. W. Kalivas and B. A. Sorg. Dept. of VCAPP, Washington State University, Pullman, WA 99164.

Activation of the hypothalamo-pituitary-adrenal (HPA) axis has been implicated in the development of psychostimulant- and stress- induced behavioral sensitization. In an attempt to further characterize the role of the HPA axis in sensitization, the effect of adrenalectomy (ADX) on the initiation and expression phases of cocaine- induced behavioral sensitization and stress- induced cross sensitization was evaluated. To determine the role of HPA axis in the initiation of behavioral sensitization, male Sprague-Dawley rats were subjected to sham surgery or ADX. A week later, rats were subjected to a sensitization paradigm with intraperitoneal injection of cocaine(Day 1- 15 mg/kg; Day 2 to 6 - 30 mg/kg). On day 7 (early withdrawal) the behavioral response to cocaine challenge (15 mg/kg) was tested. Three days later, one half of ADX rats were given corticosterone pellets and corticosterone in the night drinking water to mimic the circadian variation of corticosterone levels. After one week, rats were given a saline challenge followed by a cocaine challenge (15 mg/kg) the next day (late withdrawal). Sham controls demonstrated a sensitized locomotor response to the cocaine challenge at both early and late withdrawal times compared to the response on day 1. In contrast, sensitization was completely blocked in ADX rats at the early withdrawal time but not at late withdrawal. The effect of ADX on the expression phase of sensitization was examined by administering daily cocaine as before followed by surgery one or two days later. No effect of adrenalectomy was found on the expression of behavioral sensitization. These results suggest that corticosterone is necessary during the initiation of behavioral sensitization for the manifestation of sensitized response at the early withdrawal time but not at late withdrawal time. Similar experiments were done to examine the effect of adrenalectomy on stress- induced cross sensitization. A stress paradigm that included mild foot shock stress and restraint stress was used in place of daily cocaine injections. Preliminary results from these experiments suggest that ADX had similar effects on both cocaine- and stress induced sensitization.

766.16

ANXIOGENIC EFFECTS OF COCAINE ORAL SELF-ADMINISTRATION AND OF COCAINE WITHDRAWAL. H.M.T. Barros *, V.B. Lanziotti, S.L.Tannhauser, M.Tannhauser. Division of Pharmacology and Toxicology, Fundação Faculdade Federal de Ciências Médicas, Porto Alegre, RS, Brazil, 90050-170.

Different studies show that cocaine administration and cocaine withdrawal produce anxiogenic states in animals. This study was done to examine the behavior of rats in the elevated plus-maze during acute and prolonged cocaine self-administration and after cocaine withdrawal. Adult male rats were allowed to administer cocaine solutions (0.1 - 0.5 mg/kg) using the two-bottle choice technique during different periods of time (30 or 60 days). The amount of cocaine self-administered was in the range of 10 to 30 mg/kg/day. The animals were introduced into the elevated plus maze during the first, 15th and 30th day of cocaine administration and for 5 days after withdrawal. Ethological evaluation of frequency and duration of entrance to open and closed arms was further conducted using videotape recordings. Behavioral effects were not detected after acute oral self-administration of lower doses of cocaine. Decreased entries into the open arms was seen on the third day of treatment withdrawal. Decreased entries to the open arms was verified after acute self-administration of higher doses of cocaine . These animals also showed this behavior during the five days of testing after cocaine withdrawal.

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767.1

LIMBIC ACTIVATION DURING CUE-INDUCED COCAINE CRAVING
A.R. Childress, D. Mozley, J. Fitzgerald, M. Reivich, J. Jaggi, and C.P. O'Brien*. Depts. of Psychiatry and Radiology, Univ. of Penn. School of Medicine, and Philadelphia VA Medical Center, Philadelphia, PA 19104

Cocaine-related cues (e.g., drug users, drug talk, drug locations, drug paraphernalia, etc.) can trigger profound cocaine desire in users of the drug, but the brain correlates of this state are just beginning to be studied. The similarity of responses during cocaine craving (arousal, heart palpitations, light-headedness, ear-ringing, chest-tightness, a cocaine 'taste' in back of the throat, and even mild euphoria) to the effects of actual cocaine suggests a possible overlap of brain substrates. As many of cocaine's direct effects are mediated through the mesolimbic dopamine system, we have hypothesized activation of the same system during cue-induced craving.

To test the hypothesis of limbic activation during cue-induced craving, rCBF (regional cerebral blood flow) was imaged in abstinent cocaine patients and in matched controls without a cocaine use history during exposure to videos of both non-drug and cocaine-related scenes. Imaging of rCBF was accomplished with PET (Positron Emission Tomography) scans, using radioactively-labeled (O-15) water as the flow tracer. PET scans for each subject were co-registered with an MRI (magnetic resonance image) to permit anatomical localization of radioactivity. Cocaine patients (n=9 thus far) experienced craving to the cocaine video, and several showed rCBF increases in amygdala and in temporal pole during the cocaine video (as compared to resting baseline). Systematic activation did not occur in non-limbic comparison regions, nor in response to the non-drug cues. Control subjects (n=6) did not experience craving during the cocaine video, and did not show systematic rCBF increases to either video type. These results suggest limbic activation may be one component of cue-induced drug craving.

767.2

COCAINE-INDUCED ALTERATIONS OF BRAIN ACTIVITY IN HUMANS: AN FMRI STUDY. E.A.Stein*, J.Pankiewicz, H.H. Harsch, M. Rossing, J-K.Cho, S.A.Fuller, S.M. Rao and A.S. Bloom. Departments of Psychiatry, Pharmacology and Neurology, Medical College of Wisconsin, Milwaukee, WI 53226

Cocaine is a powerful psychostimulant agent with high abuse potential in humans. While considerable progress has been made in understanding cocaine's mechanisms of action using in vitro and animal model systems, very limited information is available on the pharmacokinetics and localization of action of cocaine in the human brain. Functional MRI is a non-invasive imaging technique that permits the visualization of functional brain activity with excellent temporal and spatial resolution without the use of ionizing radiation. Single shot echo planar imaging was obtained on a 1.5 Tesla GE Signa scanner using an insertable, balanced torque, 3-axis head gradient coil designed for rapid gradient switching. To obtain images throughout the entire brain volume, a shielded quadrature elliptical endcapped transmit/receive birdcage radio frequency coil was used. Four experienced crack/IV cocaine users received doses of cocaine IV (10, 20 and 40 mg over 60 sec) while FMR data were acquired (TR=6 sec; TE=40 msec; 8 mm slice thickness). Subjects all displayed consistent increases in heart rate, arterial blood pressure and generally reported positive subjective effects to the drug. Heterogenous activation patterns included consistent cerebral activation in the dorsolateral frontal, anterior cingulate, temporal and insular cortex. Prominant inhibitory responses were evident in several frontal fields. Signal activation latency generally varied between 1-2 min with duration of effect between 8-20 min depending upon region. These data demonstrate the ability of FMRI to map drug effects in the human brain. (Support by USPHS grant DA09465)

767.3

CEREBRAL GLUCOSE METABOLISM DURING COCAINE CRAVING: A PET FDG STUDY. Steven Grant*[1], David Newlin[1], Victor Villemagne[2], Robert L. Phillips[1], Xiang Liu[1], Alane S. Kimes[1], Carlo Contoreggi[1,3], Edythe D. London[1,4,5]. [1]Neuroimaging and Drug Action Section, Addiction Research Center, NIDA, NIH, Balto., MD 21224; [2]Dept. Pharmacol. Exp. Ther., School of Medicine, University of Maryland, Balto., MD 21201; and [3]Dept. Radiology, The Johns Hopkins School of Medicine, Balto., MD 21205.

Stimuli that are regularly associated with drug use are thought to elicit behavioral and physiological responses that contribute to addiction. In particular, relapse after abstinence may be due, in part, to cue-elicited responses. In the present study measurements of regional cerebral metabolic rates for glucose (rCMRglc) using the [F-18] fluorodeoxyglucose (FDG) method and positron emission tomography were paired with psychophysiological and self-report assessments in cocaine abusers during two experimental sessions. During the FDG uptake period, subjects were exposed to either a neutral videotape on arts and crafts or a cocaine-related stimulus complex (videotape of cocaine-related activity and paraphernalia; presence of paraphernalia and a small amount of cocaine). Analysis of data from the first 9 subjects reveals increases in self-reports of craving and overall EEG arousal during presentation of the cocaine-related stimuli. Changes in rCRMglc in response to cocaine-related stimuli differed from those observed previously in response to acute administration of cocaine. The changes in response to cocaine-related stimuli were "drug-opposite", as the stimuli caused selectively increased rCMRglc, whereas acute cocaine administration reduces glucose metabolism globally. Increases in rCMRglc occurred in regions of the prefrontal cortex, occipital cortex, the middle temporal gyrus, and the parahippocampal gyrus. The metabolic changes may reflect a distributed neuroanatomical network that mediates reactivity to cocaine-related stimuli.

767.4

DECREASED FDOPA UPTAKE IN RIGHT PUTAMEN IN PET STUDY OF COCAINE ADDICTS DURING ACUTE WITHDRAWAL. J.C. Wu*, K. Bell, E. Klein, C. Widmark, A. Najafi, C. Tang, L. LaCasse, B. Bunney, W.E. Bunney. Brain Imaging Center, UCI-CCM Psychiatry Dept., Irvine, CA 92717.

A persistent decrease in dopamine presynaptic activity has been hypothesized to be a key reason why so many cocaine addicts relapse. This study measured F-Dopa uptake, a measure of dopamine presynaptic activity, using positron emission tomography (PET) scans in nine cocaine addicts who had been drug free (off cocaine) for a minimum of one week compared with ten normal controls. Nine cocaine dependent subjects in acute withdrawal (< 30 days since last use) were studied. The cocaine dependent subjects had a mean age of 32.2 $\pm$ 14.4 years. Their mean useage of cocaine was 14.1 $\pm$ 5.2 years. The mean quantity of cocaine consumed was 1.2 $\pm$ 0.81 grams/day. The mean days off cocaine was 21.1 $\pm$ 9.5 days. All cocaine dependent subjects were male. The ten control subjects had a mean age of 36.3 $\pm$ 19.0 years. Seven control subjects were male and three were female. All control subjects had a negative urine drug screen and had a negative history of personal psychiatric illness. PET studies were performed with the University of California, Irvine (UCI) Brain Imaging Center (BIC) single ring Neuroecat IV system from CTI. Each subject received 2.0-4.0 mCi of 6-FD. FDOPA uptake was determined graphically using the method of Martin et al. (1989). FDOPA uptake was graphically represented pixel by pixel using in-house software. Images were analyzed using a method which involved dividing the striatum into a grid of 3x3 pixels. The striatal grid was subdivided into three structures (caudate, anterior putamen, and posterior putamen). Controls were significantly higher than cocaine addicts for FDOPA uptake in the right putamen. A significant structure by hemisphere by group interaction was found for FDOPA uptake activity ($F=3.53$, $d.f = 1.76, 31.59$, $p = .0466$). This finding is partly compatible with the theory presented by Dackis and Gold (1985) and Weiss et al. (1992) that there may be a chronic suppression of presynaptic dopamine activity as an adaptation to chronic dopamine exposure induced by habitual cocaine useage.

767.5

PHARMACOLOGIC MODULATION OF COCAINE-INDUCED DOPAMINE RELEASE AND LOCOMOTOR ACTIVITY: A POTENTIAL THERAPEUTIC STRATEGY FOR COCAINE ABUSE. C.-E. Chen, R. M. Straughter-Moore, D. L. Tedeschi, N. B. Russo, D. L. Alexoff, N. D. Volkow, J. S. Fowler, C. S. Chaurasia, and S. L. Dewey*. Chem and Med Dep'ts, BNL, Upton, NY 11973 & College of Pharm and Allied Health Prof, St. Johns Univ, NY 11439.

We have demonstrated that PET is a suitable imaging technique for non-invasively measuring changes in neurotransmitter concentrations following pharmacologic challenge (Dewey, et al., J. Neuroscience, 1992; 1995). Utilizing a variety of neurotransmitter-specific radiotracers, we have measured drug-induced changes in DA, 5-HT, and ACh in the primate and human brain. With our findings that certain neurotransmitters inhibit striatal DA release, combined with cocaine's ability to increase extracellular striatal DA levels, we have applied this novel strategy to an investigation of the effects of GVG (gamma vinyl-GABA, a selective suicide inhibitor of GABA-transaminase), lorazepam, and ethanol on coc-induced increases in striatal DA levels and locomotor activity using *in vivo* microdialysis in freely moving rats. In control studies, coc (20 mg/kg) increased extracellular DA levels to approximately 250 % of baseline values. GVG (100 - 300 mg/kg), lorazepam (0.1 - 0.68 mg/kg), and ethanol (1 - 3 g/kg) however, dose-dependently attenuated both this and the locomotor response. Furthermore, GVG and lorazepam produced a decrease in extracellular striatal DA levels. These findings are consistent with earlier primate PET studies using ^{11}C-raclopride and a similar challenge. Ethanol, however, had no effect on baseline striatal DA levels. Interestingly, while ethanol dose-dependently attenuated coc-induced increases in striatal DA levels similar to GVG and lorazepam, DA concentrations fell below previously established baseline levels following challenge. These studies suggest that therapeutic strategies targeted at potentiating GABAergic neurotransmission may be beneficial for the treatment of coc addiction. Supported by USDOE, OHER, NIMH 49165 and NARSAD.

767.6

SELF-ADMINISTRATION OF INTRAVENOUS COCAINE UNDER A SECOND-ORDER SCHEDULE OF REINFORCEMENT: EFFECTS OF LESIONS OF THE BASOLATERAL AMYGDALA R.B. Whitelaw, A. Markou[1], T.W. Robbins, B.J. Everitt.* Dept. of Exp. Psychology University of Cambridge, UK. CB2 3EB.[1]Scripps Res. Inst., Torrey Pines Rd, La Jolla, San Diego, CA. 92037.

In these experiments we sought to establish the intravenous (IV) self-administration (S-A) of cocaine under a second-order schedule of reinforcement in order: (i) to obtain reliable, drug-free responding for a psychomotor stimulant, and (ii) to enable investigation of the neural mechanisms by which arbitrary cues gain motivational salience and, as conditioned reinforcers, control over drug-seeking behaviour. Initially, each infusion of cocaine (1.5mg/kg IV over 4sec) was made contingent on a response on one of two identical levers and was paired with a 20s light CS. Responses on the second lever were recorded, but had no programmed consequence. When rats acquired stable rates of S-A (5 daily 2h sessions), a second-order schedule of the type FRx(FRy:S) was introduced. Priming (ie. non-contingent) infusions of cocaine were not given. Once the first infusion was obtained under the second-order schedule, further infusions were made contingent on each response (max.10 infusions /day). Each stage was repeated daily until the first infusion of each session was achieved within a 5 min criterion. Rats with bilateral, excitotoxic lesions of the basolateral amygdala (BLA) readily acquired the S-A of cocaine under a continuous reinforcement schedule, initially administering more infusions and maintaining a slightly elevated level of S-A than controls. Despite increased CS /drug pairings, BLA lesioned rats were significantly slower than controls to achieve the more demanding response requirements of the second-order schedule. Lesioned rats showed a slightly elevated dose-response curve compared to controls, but no overall shift in the inverted U-shaped function. There was also no significant difference in the locomotor response to IP cocaine (10, 20, 30 mg/kg), in drug naïve lesioned or control animals. These experiments indicate the feasibility and utility of second-order schedules in studying the neurobehavioural basis of drug S-A, and the potential importance of the BLA in the process by which previously neutral stimuli gain control over drug-seeking behaviour.

767.7

RESPONSE TO NOVELTY PREDICTS COCAINE SELF-ADMINISTRATION IN RATS. J. W. Grimm* and R. E. See. Department of Psychology, Washington State University, Pullman, WA 99164-4820.

Rats displaying a higher locomotor response to novelty than rats with a lower response have been shown to more readily acquire amphetamine self-administration. The present study sought to establish that response to novelty predicts cocaine self-administration as well. Subjects were female Sprague Dawley rats (N=10) weighing 220-280g. Response to novelty was recorded as the locomotor response of a rat in a photobeam activity chamber. The animals were then ovariectomized, implanted with subcutaneous estrogen (17-β estradiol) capsules, and fitted with intra-jugular catheters. Rats then self-administered intravenous cocaine for four days on a fixed interval schedule of reinforcement (0.219 mg/inj) and then for six days on a progressive ratio schedule of reinforcement (0.263 mg/inj). Response to novelty and responding for cocaine on both schedules was found to be highly correlated and statistically significant: response to novelty vs. 4 days FI, Pearson r=0.643, p=0.045; response to novelty vs. 6 days PR, Pearson r=0.720, p=0.019. In addition, responding for cocaine on the FI predicted responding on the PR, Pearson r=0.770, p=0.009. Thus, individual differences in cocaine self-administration can be predicted by individual differences in response to novelty in the female rat. Moreover, response to novelty predicts cocaine self-administration on a progressive ratio schedule of reinforcement, argued to be a measure of motivation to acquire a reinforcer. The relationship between response to novelty and steady state responding for cocaine and the effects of 17-β estradiol on cocaine self-administration and vaginal epithelial status will also be discussed.

767.8

CONDITIONED SUPPRESSION AS A MODEL FOR THE CLASSICALLY CONDITIONED EFFECTS OF COCAINE. C. W. Schindler*, E. B. Thorndike, M. Shoaib and S. R. Goldberg. Preclinical Pharmacol. Lab., NIH/NIDA DIR, Baltimore, MD 21224

While it is often acknowledged that the effects of drugs conditioned to environmental stimuli are important to the behavioral process of drug abuse, these processes have not been extensively studied. Further, most studies which have addressed this issue have used an environmental context as the conditioned stimuli rather that a discrete stimulus. However, this limits the types of behavioral manipulations that can be performed because of the practical issues related to the use of a diffuse stimulus. As such, our ability to study the behavioral processes involved in drug conditioning is also limited. The conditioned suppression procedure holds promise in overcoming some of these difficulties. Conditioned suppression involves the pairing of a drug injection with a discrete environmental stimulus. That stimulus-drug pairing is presented on an ongoing operant baseline and conditioning is measured as a disruption in operant performance. In the current study, rats were trained to nose-poke on a food reinforcement schedule. A 5-min tone-light compound stimulus was then presented 30 min into the session. Two min after the onset of the compound stimulus, a 3 mg/kg dose of cocaine (i.v.) was given. This dose had previously been shown to suppress food-reinforced operant behavior. After four training days, the stimulus was presented alone and shown to disrupt behavior similarly to the unconditioned drug stimulus. The tone-light compound stimulus alone did not disrupt responding. While more studies are needed to confirm the nature of the conditioned response, this preliminary data suggests that the conditioned suppression procedure may be useful in studying the behavioral procesess involved in the classically conditioned effects of cocaine.

767.9

INDIVIDUAL DIFFERENCES IN COCAINE SELF-ADMINISTRATION: DOSE-RESPONSE AND RATIO-RESPONSE STUDY. V. Deroche, F. Rougé-Pont, M. Le Moal* and P.V. Piazza., INSERM U259, Univ. de Bordeaux II, 33077 Bordeaux, France.

There are considerable individual differences among humans in drug taking behavior. Individual differences in the propensity to develop drug intake have also been evidenced in rats. When intravenous self-administration is studied with a low dose of psychostimulants, all rats self-administer the drug during the first days of testing, but in some individuals the behavior rapidly extinguishes whereas it is maintained in the others and this can be predicted by the reactivity to stressful situations: individuals with a high locomotor response to novelty (High Responders or HRs) maintain psychostimulants self-administration whereas subjects with a low response do not (Low Responders or LRs). In this report, we studied individual differences in cocaine intravenous self-administration: i) as a function of the dose of drug (1, 0.5, 0.25, 0.125, 0.06, 0.03 mg/kg/inj), ii) as a function of the ratio required to obtain an injection (1, 6, 12, 24, 36, 54, 78) at 1 mg/kg/inj. of cocaine. First rats were trained to self-administer the higher dose (7 days). The other 5 doses were consecutively tested (at least 3 days). At the training dose both LRs and HRs developed cocaine self-administration; the number of nose-pokes was significantly higher in the active hole (delivering the drug) than in the inactive one (without effect). HRs and LRs presented the classical bell-shaped dose-response curve. However for all doses, rate of responding was significantly higher in HRs than in LRs. Similar results were found when the ratio was increased. The increase of the addictive properties of cocaine in HRs can not be explained by a difference in drug pharmacokinetics, since brain levels of cocaine were not statistically different in HRs and LRs either 2, 10 or 20 minutes after an i.v. injection of 2 mg/kg of cocaine. In conclusion, individual differences in drug taking behavior can be observed in rats also when high training doses are used and do not result from a difference in drug catabolism. These data demonstrate that psychostimulant abuse results from an interaction between the drug and a specific individual substrate, inherent or acquired.

767.10

SELF-ADMINISTRATION OF COCAINE ON SECOND-ORDER SCHEDULES OF REINFORCEMENT IN RATS. **[1]R. Ranaldi*, [2]R.A. Wise and [1]D.C.S. Roberts. [1]Dept. of Psychology, Carleton Univ., Ottawa, Canada [2]CSBN, Concordia Univ., Montreal, Quebec, Canada**

Drug craving, which can trigger relapse to drug use, may be elicited by interoceptive and exteroceptive events. A rat model of cocaine addiction was used that focused on cocaine-seeking behaviour elicited interoceptively and by conditioned stimuli. Rats were housed in test chambers and had access, by pressing a lever, to a maximum of 20 intravenous cocaine injections (1.5 mg/kg/injection) beginning every morning. The rats were never primed. Although the availability of cocaine was present every morning the rats showed a self-imposed period of abstinence followed by a period of rapid consumption. Using second-order (SO) schedules of reinforcement we evaluated the role of a CS+ (a light and lever retraction) in cocaine-seeking behaviour. In a SO schedule rats must emit a number of responses to obtain a CS+ only and accumulate a number of CS+ presentations to receive a CS+-cocaine injection pairing. We compared the total number of responses that rats would emit to obtain their first and subsequent injections of the day between a group responding on a fixed-ratio (FR) schedule and a group responding on a SO schedule of reinforcement. The number of responses required per injection was incremented daily until the rats failed to consume more than 4 injections. The SO group was able to emit approximately 4 times as many responses as the FR group to obtain their first and subsequent injections. Subsequently, the effect of the CS+ on the initiation of cocaine-seeking behaviour was evaluated by comparing responding in extinction with and without the CS+. Responding in extinction was significantly greater in the presence of the CS+. The results suggest that cocaine-seeking behaviour follows a cyclical pattern and is enhanced by conditioned stimuli. Treatments for cocaine addiction may thus benefit from attempts at reducing craving elicited interoceptively and by conditioned stimuli during periods of abstinence. Funded by NIDA.

767.11

CONDITIONED PLACE PREFERENCE AND LOCOMOTION, BUT NO CONDITIONED DOPAMINE RELEASE AFTER EXPOSURE TO A COCAINE-PAIRED ENVIRONMENT. C.L. Duvauchelle*, K. Kressin, J.L. Neisewander and E. Castaneda. Lab. of Behavioral Neuroscience, Arizona State University, Tempe, AZ 85287-1104.

In order to investigate underlying biochemical correlates of cocaine-conditioned behaviors, animals implanted with chronic microdialysis guides above the nucleus accumbens were given I.V. cocaine (4.2 mg/kg) and saline in distinctly different environments. After 12 days (i.e., 6 cocaine and 6 saline injections) removeable microdialysis probes were implanted through the guides. The following day, animals were confined for 50 min to each of 3 environments: cocaine-paired, saline-paired and neutral. Dialysis samples were taken every 10 min and the session was videotaped for analysis of conditioned behaviors. Forty-eight hrs later, animals were tested for conditioned place preference (CPP). Animals exhibited CPP, conditioned locomotion and stereotyped sniffing in the cocaine-paired environment. However, there was no clear evidence for conditioned dopamine release preferential to the drug-paired compartment. These results suggest that although increased dopaminergic activity may be the driving force behind many motivated and stimulant-induced behaviors, it may not be required for the subsequent demonstration of homologous conditioned behaviors. (Supported by DA07730, DA05606 and HHMI).

767.12

A COMPARISON OF THE EFFECTS OF CONTINUOUS COCAINE INFUSIONS ON LOCOMOTOR ACTIVITY AND ON BRAIN STIMULATION REWARD. C.M. Pudiak*, R. KuoLee, & M.A. Bozarth. *Department of Psychology, University at Buffalo, Buffalo, NY 14260-4110.*

Many of the behavioral effects of cocaine show sensitization with repeated cocaine administration. This is usually demonstrated by increased behavioral responses to daily cocaine injections. However, continuous cocaine infusions have been reported to produce tolerance to some effects that show sensitization during intermittent cocaine injections. The present studies examined the effects of continuous cocaine infusions on two behaviors—locomotor activity (LMA) and brain stimulation reward (BSR).

Male, Long-Evans rats were used to examine the effects of continuously delivered cocaine infusions across 14 days of drug administration. The first study measured LMA during daily 30-min sessions. The second study determined BSR thresholds using a threshold tracking procedure: The minimum stimulation frequency necessary to maintain responding at ≥30 presses/min was determined during daily 30-min sessions in rats with lateral hypothalamic stimulating electrodes. Cocaine was continuously infused (≅30 mg/kg/day, s.c.) using osmotic minipumps (Alzet, model 2ML2). After 14 days of continuous cocaine infusions, the minipumps were removed. The development of tolerance or sensitization was further assessed by comparing responding following a cocaine injection (10 mg/kg, i.p.) 72 hrs and again 10 days after minipump removal. Responses were compared with sham operated and with unoperated control animals.

Continuous cocaine infusions increased LMA and lowered BSR thresholds across the 14-day infusion period. Both behavioral responses, however, showed tolerance with Day-1 response levels exceeding those seen on Day-14. Tolerance to the threshold-lowering effect of cocaine was much more pronounced than tolerance to cocaine's stimulation of LMA. Surprising, cocaine challenge 72 hrs and 10 days after termination of the continuous cocaine infusions failed to reveal tolerance. This suggests that unlike sensitization, tolerance seen during continuous cocaine infusions dissipates rapidly following termination of chronic cocaine administration.

767.13

ENHANCEMENT INDUCED BY TRIMETHADIONE AND PHENOBARBITAL IN COCAINE HYPERLOCOMOTOR ACTIVITY IN MICE. N. Ono*, M. Hiraki Y. Ono and T. Kuroda. Pharmacoinformatics and Medicinal Research Unit, Fac. of Pharmaceut. Sci., Fukuoka Univ., Fukuoka, 814-01, Japan.

We are investigating the influence of trimethadione, phenobarbital and diazepam on the hyperlocomortor activity produced by cocaine and methamphetamine (MAPT) in mice. Both cocaine and MAPT elicited dose-dependent hyperactivity in open field apparatus at dosages of 1-5 mg/kg and 5-10 mg/kg respectively. The hyperactivity of cocaine (10 mg/kg) and MAPT (2 mg/kg) were augmented dose-dependently by the pretreatment with phenobarbital and diazepam (1-5 mg/kg). While the hyperactivity of cocaine also was augmented by the pretreatment with trimethadione (250-500 mg/kg), but not that of MAPT. The hyperactivities of both psycho stimulants and reinforcing effects by phenobarbital were inhibited by picrotoxin, Cl^- channel blocker (0.5-1 mg/kg). In the experiment of measurement of brain biogenic amines, norepinephrine, dopamine, DOPAC, HVA, 5-HT and 5-HIAA levels were measured 30 min after cocaine by HPLC-ECD. cocaine reduced DOPAC levels in frontal cortex, but not in striatum. The reduction of DOPAC was inhibited dose-dependently by diazepam. Our data suggest that cocaine may participate in a modification of central Cl^- channel and monoaminergic system.

767.14

COCAINE-INDUCED PLACE PREFERENCES & AVERSIONS: EVIDENCE FOR AN OPPONENT-PROCESS MECHANISM OF ACTION. A. Ettenberg*, M. Raven, D. Danluck and B. Necessary. Behavioral Pharmacology Laboratory, Department of Psychology, University of California, Santa Barbara, CA 93106

Rats running an alley for IV cocaine have been reported to show a unique approach-avoidance behavior with respect to the goalbox. This ambivalence occurs even though the animals initiate runway trials normally, operantly self-administer the same dose of cocaine that they receive in the goalbox, and demonstrate place preferences for environments paired with the same dose of cocaine. To account for these paradoxical results, an opponent-process model was hypothesized in which an initial positive response to cocaine was thought to diminish in strength followed by, or concurrently with, an insurgence of an negative affective state. The present experiment tested this notion using the Conditioned Place Test in which the effects of a 0.75 mg/kg IV injection of cocaine was paired with a distinctive environment for 5 min either immediately post-injection, after a 5 min delay, or after a 15 min delay. Reliable preferences were demonstrated for the cocaine-paired environment in the no-delay condition, weaker preferences were observed in the 5 min delay condition, and significant aversions in the 15 min delay condition. These results support the notion of an opponent-process mechanism of action for IV cocaine.

767.15

DO CALCIUM CHANNELS IN THE A10 DOPAMINE REGION PLAY A ROLE IN THE DEVELOPMENT OF COCAINE-INDUCED BEHAVIORAL SENSITIZATION?. J.D. Steketee* and B.C. Braswell. Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Previous studies have suggested that L-type Ca^{2+} channels may be involved in the development of psychostimulant-induced behavioral sensitization. In particular, pretreatment with dihydropyridine Ca^{2+} channel blockers, such as nifedipine or nimodipine, blocked the development of behavioral sensitization. The mesolimbic dopamine system has been proposed to play a critical role in the development of sensitization. The mesolimbic system arises from dopamine cell bodies located in the ventral tegmental area (A10 region) to innervate numerous limbic structures, including the nucleus accumbens. The A10 region is thought to be involved in the initiation of sensitization. Thus, in these studies we examined the role of L-type Ca^{2+} channels in the A10 region on the development of sensitization to cocaine. In one set of studies, animals received daily intra-A10 pretreatment with nimodipine (10 or 30 nmol/µl, 0.5 µl/side), a Ca^{2+} channel blocker, 5 min before a peripheral cocaine injection (15 mg/kg, ip). In a second set of studies, animals received daily intra-A10 injections of Bay K 8644 (10 or 30 nmol/side), a Ca^{2+} channel opener. In both cases, animals received a challenge injection of cocaine 1 week after the last of 4 daily injections. Motor activity was monitored for 2 hr following the first daily injection and on the day of the cocaine challenge. Bay K 8644 did not induce motor activity, nor did daily injections induce behavioral sensitization to cocaine. Nimodipine did not block the acute, nor the development of the sensitized, responses to cocaine. These data suggest that the dihydropyridine sensitive Ca^{2+} channels in the A10 region do not play a role in the development of cocaine-induced behavioral sensitization.

This work was supported by grants from the Louisiana Educational Quality Support Fund (RD-A-18) and the National Institute on Drug Abuse (DA08079).

767.16

NOCTURNAL HYPOACTIVITY DURING WITHDRAWAL FROM AN ESCALATING DOSE, BINGE-LIKE ADMINISTRATION OF REPEATED COCAINE. F.J. White*, A.J. Vartanian and D.C. Cooper. Neuropsychopharmacology Lab., Dept. of Neuroscience, Finch Univ. Health Sciences/The Chicago Medical School, North Chicago, IL 60064.

During withdrawal from repeated cocaine binge administrations, human addicts experience a syndrome that includes anhedonia, anergia, depression, anxiety and cocaine craving. Both anhedonia and anxiety have been successfully induced in animal models, but there is little evidence for anergia/depression. We have tested for the presence of nocturnal hypoactivity in rats during withdrawal from an escalating dose, binge-like cocaine administration regimen. Rats received 3 injections (1 hr apart) of cocaine or saline during the light cycle for each of 5 days. Cocaine doses were as follows: days 1-2, 10 mg/kg/injection, days 3- 4, 20 mg/kg/injection and day 5, 30 mg/kg/injection. On day 6, all rats received 10 mg/kg in the early afternoon. Home-cage locomotion detectors were then used to monitor activity. In the first two experiments, cocaine treated rats (n=6/exp) were highly sensitized to the locomotor stimulating effects of cocaine as compared to age-matched saline-pretreated controls and also showed marked decreases in nocturnal locomotion during the two dark phases (12 hr cycle) of a 48 hr test. There were no differences in activity during the lights-on portion of the light cycle. In the third experiment, a third day was added to test for longevity of the effect. In this experiment, sensitization was again observed as was nocturnal hypoactivity during the first two dark periods. During the third dark period, nocturnal activity was reduced but to a lesser extent. We also noted that the cocaine treated rats exhibited significantly lower body weights at the beginning of the tests, but gained significantly more weight during the testing period. Thus, there was an apparent rebound hyperphagia along with the nocturnal hypoactivity. Supported by DA 04093 and DA 00207 to FJW.

767.17

EXCITOTOXIC LESIONS OF THE BASOLATERAL AMYGDALA ATTENUATE THE ABILITY OF DRUG ASSOCIATED CUES TO REINSTATE RESPONDING DURING WITHDRAWAL FROM SELF-ADMINISTERED COCAINE. W. M. Meil* and R. E. See. Department of Psychology, Washington State University, Pullman, WA, 99164-4820.

Environmental cues previously associated with cocaine abuse have been shown to elicit drug craving and the resumption of drug use in abstinent individuals. Despite the importance of drug associated cues in relapse, few attempts have been made to model this behavior in animals. In the present study, rats exposed to noncontingent presentation of drug paired stimuli following 14 days of 3 hr. daily cocaine self-administration sessions (0.33 mg/infusion), and 20 days of 3 hr. extinction trials showed significant reinstatement of responding. Rats placed back in the experimental chamber following 43 days of withdrawal and exposed to noncontingent presentation of drug paired cues showed higher responding than animals placed back in the chamber under extinction conditions. In addition, bilateral lesions of the basolateral amygdala (BLA), via N-Methyl-D-Aspartic acid (NMDA) microinjection (0.2 mg in 500 ul vehicle), either in the middle of or following chronic cocaine self-administration increased the rate of extinction and attenuated the ability of drug paired stimuli to reinstate responding. Lesions of the BLA following 7 days of cocaine self-administration failed to alter subsequent responding for cocaine. These results are consistent with the hypothesis that the BLA is important for the association of reinforcers with neutral stimuli, but not the reinforcing effects of cocaine itself. Furthermore, this paradigm represents a model for investigating the role of conditioned stimuli in drug craving and relapse.

767.18

MODULATION OF mRNAS FOLLOWING WITHDRAWAL FROM COCAINE AS REVEALED BY DIFFERENTIAL DISPLAY. D.J. Ennulat* and B.M. Cohen. McLean Hospital and Harvard Medical School, 115 Mill Street, Belmont, MA 02178.

We are currently using differential display reverse transcriptase PCR (DDRT-PCR) to identify genes which are regulated following withdrawal from multiple doses of cocaine. Initially the ability of DDRT-PCR to accurately and consistently reveal the regulation of known genes was investigated. Total RNA samples were isolated from the striatum of rats that had received repeated IV injections of either vehicle (0.9% saline pH 7.0) or 6 mg/kg cocaine-HCl and subjected to DDRT-PCR with primer pair 8G which is designed to amplify a 240 bp segment (8G240) of cDNA derived from *c-fos* mRNA. Independent DDRT-PCR reactions consistently indicate that the relative amount of 8G240 accurately reflects the level of expression of *c-fos* mRNA that was previously measured by Northern blot analysis [Ennulat *et al.*, (1994), Mol. Brain Res. 26:106-112.]. Here DDRT-PCR mediated amplification of known genes has the advantage of providing an internal control to confirm the validity of the DDRT-PCR reactions. An anonymous PCR product (8G168) was recently observed to be induced at 1 day following the last cocaine treatment, but not at 1 hr. Two other PCR products (8G132 and 8G127) are reproducibly repressed in cocaine treated animals both 1 hr. and 1 day after the last exposure to cocaine. Further characterization of these PCR products is ongoing and additional screening with other selected primer pairs is expected to reveal additional candidate genes. This work is supported by grants from NIMH and NARSAD.

767.19

CHANGES IN Na+ CHANNEL FUNCTION DURING WITHDRAWAL FROM CONTINUOUS OR INTERMITTENT COCAINE ADMINISTRATION. G.R. King, and E.H. Ellinwood Jr.*, Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710

Previous research clearly indicates that intermittent cocaine administration induces sensitization, while continuous cocaine administration induces tolerance, to some of the effects of subsequent cocaine administration. Much research has focussed on the uptake inhibiting properties of cocaine, in spite of the fact that cocaine is a potent local anesthetic. The present experiments evaluated whether sensitization and tolerance are related to functional changes in Na+ channels. Rats were pre-treated with 40 mg/kg/day cocaine for 14 days by either subcutaneous injections or osmotic minipumps. On day 7 of withdrawal the rats were sacrificed, and striatal slices obtained. The slices were perfused with 35 mM K+, to induce DA release, in the absence and presence of 0, 5, 10 or 20 μM procaine, a local anesthetic and Na+ channel blocker. The results indicated that in slices from the intermittent administration rats, the ability of procaine to inhibit K+ induced release was attenuated. In contrast, in slices from the continuous administration rats, the ability of procaine to inhibit K+ induced release was enhanced.

EPILEPSY: ANIMAL MODELS III

768.1

SEIZURE SUSCEPTIBILITY IN AN ANIMAL MODEL OF NEURONAL MIGRATION DISORDERS. S.C. Baraban* and P.A. Schwartzkroin, Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

We investigated the effect of prenatal (E15) methylazoxymethanol acetate (MAMac), a teratogenic agent, on seizure susceptibility of the offspring Cresyl violet-stained sections of hippocampus confirmed the presence of ectopic pyramidal cells in s.radiatum and s.oriens of region CA1/CA2. In awake, freely-behaving animals (P60) from MAMac-injected dams, the latencies to flurothyl-induced seizure activity (myoclonic jerk: 173 ± 2.3 s; forelimb clonus: 215 ± 4.6 s) were significantly shorter than those of age-, weight- and sex-matched controls (200 ± 6.9 s and 238 ± 8.8 s, respectively). Shorter seizure latencies were associated with larger numbers of ectopic pyramidal cells. *In vitro* intracellular recordings from CA1 pyramidal cells in MAMac-treated tissue (P25-P35) were similar in many of their intrinsic properties (e.g., RMP, AP amplitude, R_{in}) to cells from control tissue. The synaptic responses of CA1 cells in MAMac and control hippocampus were also comparable. However, MAMac-treated tissue could be distinguished as having an unusually high proportion of CA1 cells (>60%) which fired a burst of action potentials in response to suprathreshold current injection. Further, elevation of extracellular $[K^+]_o$ from 3, to 6 mM resulted in evoked epileptiform discharge activity (10/10) and spontaneous epileptiform activity (8/13) in slices from MAMac-treated rats; such epileptiform activity was rarely observed in controls (evoked: 3/9; spontaneous: 0/8). These data suggest that prenatal MAMac administration results in functional epileptogenic abnormalities characterized by reduced seizure thresholds. As such, the MAMac model may serve as a useful model for studying early onset epilepsies resulting from abnormal neuronal migration. This work was supported by NIH grant NS15317.

768.2

FUNCTIONAL CHARACTERIZATION OF THE *JERKY* PROTEIN IN MICE. G.P. Donovan* and M. Toth. Department of Pharmacology, Cornell University Medical College, New York, NY 10021.

We have identified a mouse gene named *jerky* that, when disrupted by insertional mutation, causes an epileptic phenotype and EEG. In order to characterize the mechanism by which epileptogenesis occurs, it is necessary to do both localization studies as well as functional analysis of the *jerky* protein. Computer database searches have revealed significant homology to sequences represented in centromere binding protein-B (CENP-B), Drosophila transposable element POGO-R11, and yeast regulatory proteins RAG3 and PDC2. The pairwise alignments were most significant with CENP-B and POGO-R11 (P-values < 10^{-16}). The order of the domains was identical in all sequences, further suggesting the relationship between them. In addition to these homologies, all four of these proteins have DNA binding characteristics. In order to determine if the *jerky* protein also has this function, we have purified the 41.7 kD *jerky* protein using a 6-His fusion vector system. We have also epitope tagged the *jerky* protein at the N-terminus and transfected this construct into mammalian cells for immunohistochemical analysis and further functional studies.

768.3

DISSECTING THE COMPLEX GENETICS OF EPILEPSY IN THE EL INBRED MOUSE STRAIN W.N. Frankel*, C.M. Lutz, E.W. Johnson, A. Valenzuela The Jackson Laboratory, Bar Harbor, ME

In the EL mouse strain, several genetic loci combine to produce recurrent, tonic-clonic and generalized seizures like common epilepsies in humans. In the mice, seizures occur naturally at ~90 days of age, although for genetic studies we induce at ~30 d. by gentle rhythmic stimulation to measure seizures quantitatively. Genetic crosses to date reveal several loci: *El5* (Chr 14), *El2* (Chr 2) *El1* (Chr 9), *El3* (Chr 10) and two provisional loci *El4* (Chr 9) and *El6* (Chr 11). Effects of these loci depend not only on strain background but also on the *kind* of cross. In a backcross with ABP, *El2*, *El1* and *El3* account for most of the trait. But only the minor *El3* had an effect in the corresponding intercross. In an intercross with the DDY strain, a close relative to EL, *none* of these loci had major effects. The big difference between this pair, *El5*, maps to Chr 14 accounting for ~35% of the genetic variance. Several minor players were found, including possibly *El1*, and only these were seen in a corresponding backcross to DDY. Finally, in (EL x C3H)F2 intercross progeny, *no* known *El* locus was detectable.

Such complexities are enlightening and disturbing. First, they imply there is no single *El* locus 'essential' for high seizure frequency. Second, although many loci can influence seizures, to explain strain-*independent* effects these loci must exhibit epistatic interactions. While complex, however, the results are still reproducible within a genetic context. Thus it is possible to not only identify candidates, but to *de novo* positionally clone loci with large effects by using special mouse strains. For example, we constructed and tested ABP.EL-*El1*, *El2* and *El3 congenic* strains. Compared to ABP, ABP.EL-$El2^e$ strain seizes much more, ABP.EL-$El1^e$ seizes slightly more, and ABP.EL-$El3^e$ no more than ABP. These results are consistent with the relative effects of these loci in prior crosses. We are now testing derivative recombinant strains to find *El2*, and in similar studies, *El5*.

768.4

EPILEPTIC MICE SHOW DEVELOPMENTAL DIFFERENCES FOR VSCC SUBTYPES. M.J. Litzinger*, R. Hardy, J. Speakman, J.R. Abbott & S.K. Jensen. Lab. of Applied Neurobiology, Depts. of Pediatrics and Physiology, University of Utah. Salt Lake City, Utah 84132.

Developmental differences in voltage sensitive calcium channel (VSCC) subtypes have been discovered in Swiss Webster mouse brain cortical preparations using displacement binding of ω-conotixin(CgTx) GVIA by ω-CgTx MVIIA (Abbott & Litzinger, 1994). Abbott and Litzinger suggest the presence of a juvenile form of the N-type calcium channel in the developing mouse brain. This juvenile form seems to disappear after dendro-axonal synapse formation during the "critical period". Esplin, et al (1994) showed that the DBA epileptic mouse whole brain had a different developmental sequence of ω-CgTxGVIA binding. DBA mouse cortex showed the same unusual binding pattern(Jensen, et al, submitted,1995). The potential for abberant synapse formation in in the DBA mouse brain was proposed.

The present study compares displacement data from the developing DBA mouse whole brain to Swiss Webster mouse whole brain. At postnatal days 4, 8 and 16, ω CgTx GVIA was displaced by ω-CgTx M VIIA. Unlike the Swiss Webster data, the DBA mouse showed no juvenile subclasses of the N-type channel at day 8 or day 16. However, preliminary data suggest that thef juvenile subtype channel is seen earlier on day 4 and is consistant with the different developmetal profile shown in the DBA mouse whole brain ω-CgTx GVIA binding!

Neurodevelopment of epileptic DBA mice is clearly different compared to Swiss Webster mice when following markers of presynaptic calcium channels. Perhaps these developmental differences in the presynaptic calcium channels affect the release of neurotransmitter in the DBA mouse.

768.5

DISINHIBITION OF THE DENTATE GYRUS FROM THE EPILEPSY PRONE EL MOUSE REVEALS PAROXYSMAL FIELD DISCHARGES. H. Wang*, W. Frankel and L.M. Masukawa, Dept. of Neurology, University of Pennsylvania Medical School and The Graduate Hospital Research Center, Philadelphia, PA 19146 and The Jackson Laboratory, Bar Harbor, ME 04609.

A genetic model of epilepsy, the epilepsy prone EL mouse, develops spontaneous seizures after approximately 90 days of life. Hippocampal brain slices from EL mice were examined during a period of 8-14 weeks of age. Field responses from the granule cell layer of the dentate gyrus during perforant path stimulation were compared with those from the genetic control, the DDY mouse strain. In control solution, the orthodromic and antidromic responses were similar for the two mouse strains, each response type was characterized by a single population spike, and the presence of a positive field PSP (duration: 10-20 ms) or an absence of a field PSP, respectively. Paired pulse depression recorded at 10, 20 and 70 ms interstimulus interval was significantly less in the EL than the DDY mouse. After exposure to bicuculline (10 μM) for a period of 40 minutes or more, the orthodromic response was distinctly different in the two strains. The response in the DDY mouse (6/6) exhibited multiple population spikes (2-6) superimposed on a more prolonged monophasic PSP (duration: 30 ms), whereas, the response in the EL mouse (9/9) was greatly prolonged (> 400 ms) and was often biphasic with a large, protracted negative component following an initial positive potential (6/9). During the initial 150 ms of the paroxysmal-like response, a rapid population spike discharge (10-50 spikes) occurred. Therefore, the orthodromic response of the dentate gyrus examined in EL mice during a period somewhat before the period of spontaneous seizures was normal in appearance but exhibited reduced feedback inhibition. During disinhibition an underlying paroxysmal excitatory response was revealed which might function later in life during spontaneous seizure generation. Supported by NIH grant # NS23077 to LMM.

768.6

SELF - SUSTAINING STATUS EPILEPTICUS RESULTING FROM BRIEF PERFORANT PATH STIMULATION IN FREE MOVING RATS. A.Mazarati, Y.Shirasaka*, C.G.Wasterlain, VA Medical Center Sepulveda CA 91343-2099, Dept. of Neurology and Brain Research Institute, UCLA School of Medicine.

We studied the effects of perforant path stimulation (PPS) of various durations on the induction of self- sustaining status epilepticus (SSSE), changes in paired- pulse inhibition and brain damage.

Male Wistar rats (13 -14 weeks old) were implanted with a stimulating electrode into the perforant path and a recording electrode into the dentate gyrus and stimulated in the awake state four weeks after surgery (one per minute 10 s trains of single stimuli at 20 Hz, 20 V, 0.1 ms).

After 30 min of PPS, 9 of 10 rats had SSSE lasting at least 3 h and the last rat displayed seizures by EEG without overt behavioral convulsions. 4 rats died within 24 h after PPS. The 5 animals recorded displayed paroxysmal EEG activity and some decrease of paired- pulse inhibition 3 days after PPS. 7 rats had severe bilateral hippocampal damage and lesions in amygdala, pyriform, entorhinal and neocortex, 3 had mild or minimal injury. The degree of brain damage correlated with the severity of SSSE. 15 min of stimulation induced motor SSSE in two of 3 rats and interictal spikes in 1 animal. 1 animal died 24 h after PPS, 1 rat displayed paired- pulse disinhibition 3 days after PPS and in 1 animal paired- pulse inhibition recovered at this time. Neuronal injury was observed bilaterally in hilus, CA1 and CA3. 7 min of PPS did not cause SSSE, loss of paired- pulse inhibition or significant cell loss in any of 4 rats. These results demonstrate that brief PPS is sufficient to trigger SSSE, loss of inhibition and hippocampal damage.

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768.7

THE ROLE OF $GABA_A$ LOW-AFFINITY RECEPTORS IN CONTROL OF SEIZURES. J. Velíšková, L. Velíšek, M.L. Nunes and S.L. Moshé*, Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

GABAergic transmission in the substantia nigra pars reticulata (SNR) controls seizure propagation. In adult rats there are two discrete regions which mediate opposing effects on seizures. Bilateral microinfusions of muscimol (both high- and low-affinity $GABA_A$ receptor agonist) into anterior part of SNR are anticonvulsant, while the infusions into the posterior-lateral part are proconvulsant. In contrast, in 15-day-old rat pups, only the proconvulsant muscimol-sensitive network is present.

In this study, we determined the role of low-affinity $GABA_A$ receptors in the SNR in the control of seizures. In 15 day old rats, bilateral intranigral infusions of ZAPA (a low-affinity $GABA_A$ receptor agonist; 2 μg/0.25 μl) significantly increased the latency to onset of both clonic and tonic-clonic flurothyl-induced seizures. In the adult rats, the same doses of ZAPA had no significant effects on either clonic or tonic-clonic flurothyl-induced seizures in either anterior or posterior part of the SNR compared to the saline-infused controls.

The data suggest that the low-affinity $GABA_A$ receptors in SNR are involved in control of clonic and tonic-clonic seizures in 15-day-old rats, but not in adults.

768.8

SELECTIVE CHANGES IN GENE EXPRESSION ASSOCIATED WITH EPILEPTOGENESIS IN HIPPOCAMPAL/ENTORHINAL CORTICAL SLICES RS Vick, A Rafiq, DA Coulter, ER Jakoi, and RJ DeLorenzo* Department of Neurology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298

Long term exposure of hippocampal slice preparations to extracelular 0 Mg^{2+} has been shown to produce recurrent seizures. This study investigated changes in specific mRNA levels that occur in association with these recurrent seizures. Hippocampal/entorhinal cortices (HEC) from male 21-30 d rats were removed, sliced, and placed in oxygenated artificial cerebrospinal fluid (ACSF) in the presence or absence of Mg^{2+} for 2 h. The slices were returned to ACSF containing Mg^{2+} for 3 h. Epileptogenic activity was was elicited throughout representative HEC preparations by a single electrical stimulation. Corresponding slices were frozen, blocked, and cryosectioned for in situ hybridization (Perlin et al., *PNAS*, 90:1741, 1992). Complementary oligonucleotide probes to the $GABA_A$ α1 and α2 subunit, ligatin, α CAMKinase II, and the NMDA receptor subunits NR1, NR2a-c were 3' end-labeled with [a-[^{35}S]thio]dCTP. Autoradiography was analyzed by a Jandel-Mocha image analysis system. In situ hybridization demonstrated that levels of $GABA_A$ α2 and ligatin mRNA decreased in the CA1-2, CA3-4, and DG regions. Levels of $GABA_A$ α1 and α CAMKinase II mRNA did not change throughout the HEC while levels of the NMDA subunits (NR1, NR2a-c) mRNA increased. Decreases in the levels of mRNA for $GABA_A$ α2 and ligatin and increases in the levels of mRNA of the NMDA subunits for at least 3 h post 0 Mg^{2+} exposure are consistent with the hypothesis that long term gene changes are induced during epileptogenesis and may contribute to altered neuronal excitability.

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768.9

DECREASED CALCIUM-CALMODULIN KINASE II IMMUNOREACTIVITY IN HIPPOCAMPAL CA1 NEURONS IN AN ELECTRICAL STIMULATION MODEL OF STATUS EPILEPTICUS. A.C. Rice*[1], S.B. Churn[1], V.J. Obias[2], and R.J. DeLorenzo[1], [1]Department of Neurology, [2]Department of Physiology, Virginia Commonwealth University, Richmond, Virginia 23298-0599

The well characterized hippocampal damage observed following status epilepticus (SE) involves cell loss in the dentate hilar region, and the CA1 and CA3 pyramidal cell layers. The biochemical mechanisms underlying the cell death are hypothesized to be related to alterations in calcium homeostasis. Calcium-calmodulin kinase II(CaM kinase II) is a calcium dependent kinase involved in many cellular processes such as neurotransmitter release and cytoarchitecture formation. Using an ischemia model, this lab has demonstrated a decrease in CaM kinase II activity which corresponded to a decrease in CaM kinase II-β (60 kDa subunit) immunoreactivity in hippocampal regions with subsequent cell death. Since CaM kinase II activity also decreased in the continuous hippocampal stimulation(CHS) model of SE, we examined the localization of CaM kinase II-β immunoreactivity in CHS treated animals. The CHS paradigm involves 90 min of electrical stimulation (400μAmps, 50Hz in 10s trains every 11s). At 4 hr ,and 3 and 7 days post-CHS the animals were paraformaldehyde perfused and brain sections were paraffin embedded. Ten micron sections were incubated with anti-CaM kinase II-β monoclonal antibodies (1:500 dilution), followed by an ABC elite kit (Vector Labs) protocol and developed using diaminobenzamidine and hydrogen peroxide. Positively staining cells were counted per unit length in the CA1 region. Compared to surgical controls the CHS treated animals had decreased (approximately 40%) immunoreactive cells at all time points examined. Loss of immunoreactivity at the early time points without cell loss indicates that the loss of CaM kinase II predicts cells undergoing delayed neuronal cell death.

768.10

Novel Populations of Calbindin-D28k Immunoreactive Neurons in the Hippocampus Following Tetanus Toxin Induced Seizures In Early-Life. Chong L. Lee*, and John W. Swann, The Cain Foundation Laboratories, Dept. of Pediatrics, Baylor College of Medicine, Houston, TX.

Early-life seizures induced by tetanus toxin (TT), produce novel populations of calbindin-D28k immunoreactive (CAL-ir) neurons in the hippocampus. While CAL staining is reduced in CA2 and the dentate granule cell (GCL), novel populations of CAL-ir neurons are observed in the alveus-stratum oriens (A-SO) of CA1 and the hilus. To better under-stand the origin of these CAL-ir neurons, rat pups (PND 3-20) were given daily IP injection of bromodeoxyuridine (BrdU - 50 mg/kg), for 1-20 days. On PND 10, TT (2.5 ng) was injected into the right CA3 subfield and animals monitored daily for 1 hr for occurrence of behavioral seizures. On PND 54-60, animals were processed for double immunohistochemistry to reveal colocalization of BrdU, CAL and somatostatin (SRIF) in the dentate gyrus. Double labeled neurons were not observed in CA1. Dense BrdU-ir granule cells (GC), double labeled with CAL were observed in the GCL. Many (but not all) of the novel hilar CAL-ir neurons were double labeled with BrdU and had soma size similar to double labeled GC in the GCL. BrdU and SRIF double labeled neurons was rarely observed in the DG. No CAL and SRIF double labeled neurons were encountered. In summary, the population of CAL-ir interneurons in the A-SO of CA1 may represent novel expression of CAL by these intrinsic neurons. However, the new population of CAL-ir neurons in the hilus are born postnatally (BrdU-ir) and may represent displaced GC. Both populations could contribute to the change in the excitability recorded in the hippocampus. (Supported by NIH Grant NS18309)

768.11

HYPOXIA-INDUCED SEIZURE ACTIVITY PRODUCES MORPHOLOGICAL AND FUNCTIONAL CHANGES IN THE DEVELOPING HIPPOCAMPUS. J. Owens, Jr., C.A. Robbins, H.J. Wenzel, and P.A. Schwartzkroin*. Depts. of Physiology/Biophysics and Neurosurgery, Univ. of Washington, Seattle, WA 98195.

Adults with temporal lobe epilepsy are more likely to have experienced seizures in childhood than are adults without epilepsy. Yet it remains unclear whether the primary pathogenetic factor underlying subsequent epilepsy in adulthood is: a) the event or condition which provoked the childhood seizures; b) the seizures themselves; or c) an interaction between the two. Perinatal hypoxia-ischemia is a frequent antecedent of pediatric seizures and a not uncommon obstetrical complication. In order to separate the effects of hypoxia-induced seizures from hypoxia alone, we exposed 10 day old rats to one of two hypoxia protocols: 1) 60 minutes of approximately 5% oxygen followed by one hypoxia-induced seizure or 2) 60 minutes of hypoxia during which the oxygen concentration was manipulated to produce 8 to 12 seizures. Immediate mortality was higher in the single seizure group (8%) than in the multiple seizure group (<1%). Extracellular recordings from hippocampal slices prepared 3 days post-hypoxia showed an increased gain (relative to controls) in the input-output curve only for animals from the multiple seizure group. Analysis of cresyl violet stained sections through the dorsal hippocampus revealed a significant increase, relative to littermate controls, in both the number and the density of pyknotic cells in the stratum granulosum and hilus of the dentate gyrus in animals from the multiple seizure group but not from the single seizure group. Flurothyl testing 60 to 80 days after the hypoxic exposure demonstrated a significant decrease in latency to the first myoclonic jerk and to forelimb clonus in animals from the multiple seizure group, while a lesser reduction was evident in animals from the single seizure group. These results suggest that the seizure episodes contribute significantly to the morphological and functional changes seen in post-hypoxic hippocampus. (Supported by NIH, NINDS grant# 15317)

768.12

THALAMIC CONTROL OF HIGH VOLTAGE SPIKE-AND-WAVE SPINDLES IN RATS. G. Marini*, G.Macchi, M.Mancia (SPON: European Neuroscience Association) Istituto di Fisiologia Umana II, Università degli Studi; *INB-CNR, Milano; Clinica Neurologica, Università Cattolica, Roma (Italy).

It has been shown that the thalamic reticular nucleus (NRT) exerts a control over neocortical paroxysmal activity. Unilateral electrolytical and chemical lesions of the NRT released spike-and-wave complexes on both cerebral hemispheres, but primarily on the contralateral one, in freely moving Sprague Dawley rats. Our working hypothesis is that these paroxysms may be induced by the disinhibition of the contralateral NRT and then transferred via the corpus callosum to the other hemisphere. Four rats, previously implanted for chronical recording of EEG and nuchal EMG, were ketamine-sedated and received ibotenic acid injections stereotaxically placed in the left NRT. The corpus callosum was then transected with a small surgical blade mounted on a stereotaxic holder.The day after the injection high-voltage spike-wave spindles (HVS) at 6-9Hz occurred associated with awake immobility on the right hemisphere. The discharges arose from a desynchronized EEG activity and were not apparent during sleep. The duration ranged from 2 to 6.5s. The intraepisodic frequency of these HVS was 5-10 s. Pinching of the tail interrupted the ongoing HVS. The asymmetry in the two hemispheres was also corroborated by the spectral analysis. The ipsilateral EEG displayed oscillations within a frequency band of 0.5-7Hz while the contralateral one showed the burst of activity in the bands 4-7 and 7-14 Hz. HVS are not present spontaneously in this strain of rodent species. Since in rats HVS patterns are viewed as epileptic phenomena because of their spontaneous, episodic, recurrent, and paroxysmal nature, their appearance after callosotomy only on the contralesioned hemisphere support the idea that the bilaterality of the paroxysm is due to callosal projections and that the disinhibition of the NRT contralateral to the lesion may underlie epileptiform patterns.

768.13

DEXTRAN PRESERVATION OF CA3, CA4 AND DENTATE IN HIPPOCAMPAL BRAIN SLICES. **H.Qi*, F.E.Hospod, S.Motwani, S.Trowbridge, C.S.Patlak , G.C.Newman. Depts. of Neurology and Surgery , SUNY at Stony Brook, NY and VAMC at Northport, NY.**

Brain slice models of epilepsy often focus on CA3 and dentate neurons yet hippocampal slices from adult rats show severe neuronal injury in both areas if incubated submerged in Krebs-Ringer (K-R) at 37°C. In an effort to reduce brain slice swelling during incubations, we have re-considered the use of dextran which was shown over 20 years ago to eliminate swelling. We have found that modifying K-R by adding 3% dextran and reducing NaCl by 16 mM significantly improves both histology and water gain.

Slices were incubated for 4 h *in vitro*, submerged 1 mm at 37°C with 95%O_2-5%CO_2. Dextran improves histologic score (1 - 5; lower is better) in all slice regions, but especially in CA3 (1.9±0.2 *v.* 3.3±1.2) and dentate (1.3±0.7 *v.* 2.8±0.6). Wet weight / protein falls from 14.4±3.0 in K-R to 11.9±1.8 with dextran. Water gain over 4 h falls from 33% to 9%. There is no change in tissue ATP, ADP, AMP or energy charge, in the relative size of the extracellular space (0.25) or the diffusion coefficients for either ^{14}C-PEG(4000) (3.8x10^{-7} cm^2 sec^{-1}) or ^3H_2O (1.2x10^{-5}). Dextran increases glucose utilization from 39.7±11.3 to 78.3±19.5 μmole/100g/min.

Thus, dextran markedly improves histology and reduces water gain of adult rat hippocampal brain slices incubated slightly submerged at 37°C. This is not due to improved adenylates or changes in diffusion but is associated with increased glucose utilization. Dextran should be considered when neuronal instability or obvious water gain is encountered in brain slices.

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768.14

SEIZURE TESTING AFTER GLOBAL ISCHEMIA DOES NOT INDUCE HEARING LOSS IN POST-ISCHEMIC AUDIOGENIC SEIZURE-PRONE (PIAS) RATS. K. H. Reid*, C. Young and V. Iyer Depts. of Anat. Sci. Neurobiol. and Neurology, Univ. of Louisville, Louisville KY 40292.

PIAS rats show some reduction of seizure susceptibility with time; this varies greatly between rats (Kawai et al., Epilepsia 33:38,1992; Reid et al., FASEB J. 8:A660,1994). It has been suggested (Naritoku et al., Exp. Neurol. 115:317,1992) that the repetition of intense auditory stimulation could induce a hearing loss which would appear as a loss of sensitivity to sound-triggered seizures. Hearing impairment has been shown to contribute to the difference in seizure sensitivity between GEPR3 and GEPR9 rats (Faingold et al., Exp. Neurol. 99:678,1988). We used auditory evoked potentials to evaluate hearing in 7 post-ischemic rats repeatedly during seizure testing with a 110 dB alarm bell, and found no hearing loss as measured by threshold to click stimuli at 10/sec. (BAEPs, average of 1024 responses). We conclude that some other process - possibly a return of GABAergic inhibition (Penix et al., Neurol. 43:A312,1993), neuronal regrowth or CNS reorganization - underlies recovery from seizure susceptibility in PIAS rats.

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768.15

ABERRANT NEURONAL FIRING PATTERNS IN DEEP LAYERS OF SUPERIOR COLLICULUS SUBSERVE AUDIOGENIC SEIZURE EXPRESSION IN GENETICALLY EPILEPSY-PRONE RATS

M.E. Randall and C.L. Faingold*. Dept. Pharmacology, Southern Illinois Univ. School of Medicine, Springfield, IL 62794.

The deep layers of superior colliculus (DLSC) have previously been implicated in the neuronal network for audiogenic seizures (AGS) in the genetically epilepsy-prone rat (GEPR-9). The present study examined DLSC neuronal responses using chronically implanted microelectrodes. Normal Sprague-Dawley rats and the GEPR-9 were anesthetized with ketamine/xylazine (85/3 mg/kg), and microwire electrodes were implanted into DLSC. Unit activity was recorded at least one week later in freely-moving rats. Responses were evaluated to step-wise acoustic intensity increments up to 100 dB SPL (12 kHz tone bursts, 100 msec, 1/2sec), effective in inducing AGS at high intensities. DLSC neurons in the GEPR-9 exhibited a precipitous onset of acoustically-evoked firing at 80-90 dB, N=16), which was a considerably higher intensity than that observed for the more gradual onset of neuronal responses in normals (at 65-75 dB). In most DLSC neurons rapid tonic firing began 1-2 sec before the onset of wild running, and it continued unchanged during AGS. DLSC firing ceased as the post-ictal period began. The DLSC is proposed to be a major nucleus in the trajectory of the AGS network from inferior colliculus (IC) to brainstem reticular formation (RF). The aberrant response patterns in GEPR-9 DLSC neurons share certain similarities with IC (threshold elevation, post-ictal silence) and others with RF neurons (precipitous onset). The aberrant pattern of firing changes in DLSC is unique and suggests that the DLSC may be critical in AGS, particularly in generation of the wild running component.

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768.16

STIMULUS INTENSITY AND GENDER DEPENDENT CHARACTERISTICS OF AUDIOGENIC SEIZURE ACTIVITY IN RAT. K.C. Ross[1], M.H. Durkin[1], L.A. Abercrombie[1] and J.R. Coleman[1,2]*. Depts. of Psychology[1] and Physiology[2], Univ. of South Carolina, Columbia, SC 29208.

Audiogenic seizure (AGS) activity in rats results from priming to a high intensity sound during an early sensitive period. One goal of this study was to assess later seizure activity patterns associated with exposure to different sound intensity levels. Previous AGS research has utilized dB levels ranging from 98 dB SPL (Frye et al., 1986) to over 120 dB SPL (Snyder-Keller and Pierson, 1992), which may elicit differential AGS activity. L-E rats at postnatal day 14 (PND 14) were exposed to 10 KHz tone bursts for 8 m. at 120 dB SPL. Beginning at PND 24, subjects (n=16) were tested for AGS using continuous white noise at four intensity levels: 90 dB, 100 dB, 110 dB, and 120 dB, and were recorded as displaying no seizure activity, wild running only, or wild running followed by clonus. There was a significant main effect of intensity ($p<.000$). Post-hoc analysis revealed significant pairwise differences in AGS activity between 100 dB and 110 dB ($p<.000$) and between 110 dB and 120 dB ($p<.000$). The main effect of gender and the interaction of intensity by gender was not significant.

Another goal of this study was to explore gender differences with regard to seizure onset latency and onset duration. Onset latency for males (M=36.73 s, SD=12.09) was longer than that for females (M=31.81 s, SD=14.36; $p<.06$). The difference in onset duration for males (M=23.70 s, SD=9.50) and females (M=24.33 s, SD=6.86) was not significant. These data suggest first that AGS activity is intensity-dependent, with 120 dB always eliciting clonus. Secondly, there appear to be some gender differences in AGS characteristics.

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768.17

COMPARISON OF ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONS IN SUBNUCLEI OF RAT INFERIOR COLLICULUS (IC) IN VITRO. Y. Li*, M.S. Evans, D.N. Chakravarty, and C.L. Faingold, Depts. Pharmacol. Neurol. Southern Illinois University School of Medicine, Springfield, IL 62794.

The major subnuclei of IC play disparate roles in processing of acoustic stimuli and differential roles in audiogenic seizures. The basis for the differing roles of these subnuclei may involve differences in synaptic responses and/or differences in membrane properties of the neurons. The present study examined this issue using intracellular recordings from neurons from external cortex (ICx, N=11), dorsal cortex (ICd, N=32) and central nucleus of IC (ICc, N=12). The locations of the IC subnuclei were determined according to a standard stereotaxic atlas (Paxinos and Watson, 1986). Coronal slices (400 μm) of IC were prepared using a vibratome. Mean resting membrane potential was similar in all subdivisions [61±12mV, SD (ICx), 67±8mV (ICd) and 66±8 (ICc)]. Input resistance measured in each subdivision differed [66±29 MΩ (ICx), 49±21 (ICd) and 33±14 (ICc)]. The mean amplitudes of the action potentials (APs) of cells within the nuclei was similar but the mean AP widths differed. Thus, AP amplitude in ICx cells was 67±9mV, width at ½ height of 0.94±0.37ms. The mean AP amplitude of ICd cells was 68±21mV, width at ½ height of 0.71±0.4ms. The mean amplitude of APs of ICc cells was 70±12mV, width at ½ height of 0.37±0.14ms. Depolarization revealed a calcium mediated "hump" at the onset of the I-V curve deflection in ICd cells that was not seen in ICx or ICc cells. Spontaneous action potentials at resting membrane potential were seen more frequently in ICx cells than in ICd and ICc cells. Afterhyperpolarization was also less common in ICx neurons. Stimulation in CoIC elicited a single AP followed by a late depolarization (N=9 of 12). CoIC stimulation did not elicit a response in ICc neurons. However, stimulation of the lateral lemniscus elicited an action potential followed by a hyperpolarization and a slow return to resting potential in ICc and ICd cells. The present study supports the concept that differences in membrane physiology and responses to synaptic input of neurons in these IC subdivisions may make significant contributions to the different roles in acoustic processing and in the network for audiogenic seizures. (Support NIH NINDS NS 21281)

768.18

INDUCTION OF INTERLEUKIN-1α IN THE HYPOTHALAMUS OF THE FRINGS AUDIOGENIC SEIZURE SUSCEPTIBLE MOUSE . S.L. Skradski[1], L.K. Jackson[2], H.H. Wolf*[1], H.S. White[1] and L.C. Gahring[2], Anticonvulsant Drug Development Program; Depts. Pharmacology and Toxicology[1] and Medicine[2], Univ. Utah, and S.L.C. VA-GRECC[2], S.L.C., UT.

The cytokine interleukin-1α (IL-1α) is a mediator of inflammation and immunity in the peripheral immune system and also a mediator of CNS function such as slow wave sleep and fever. IL-1α can be induced in the CNS by stimulation of the periphery with inflammatory agents such as bacterial lipopolysaccharide and also by seizure inducing convulsants such as kainic acid. Kainic acid induces lesions and immune cell infiltration, which complicate the interpretation of these results. Therefore, we chose to determine the effects of seizures on CNS cytokine production following sound stimulation. The Frings mouse displays generalized tonic-clonic seizures upon exposure to high intensity sound stimulus (110 db, 11kHz). To determine if transcription of IL-1α mRNA is altered by seizure induction, Frings mice were exposed to sound stimuli, observed to undergo tonic extension seizure, sacrificed and perfused with saline (to remove blood contamination) either immediately, 2, 4, 6 or 24 hrs post-seizure. RNA from tissue samples was extracted and RT-PCR performed using IL-1α primers. The results were quantified by measuring EtBr incorporation into the PCR product. IL-1α mRNA levels were elevated in the hypothalamus of 1 of 3 mice sacrificed at 4 hrs and 2 of 3 mice sacrificed at 6 hrs post-seizure. IL-1α mRNA levels returned to normal levels by 24 hrs post-seizure. The induction of IL-1α was blocked completely in mice pre-treated with the anti-inflammatory glucocorticoid, dexamethasone. These results suggest that IL-1α plays a role in post-ictal alterations in the CNS. *Supported by NIH contract NO1-NS-4-2311 and NIH grants AG04418 , NS0990, and the American Federation for Aging Research.*

EPILEPSY: PRIMATE STUDIES

769.1

SPONTANEOUSLY APPEARING SHARP FIELD POTENTIALS IN HUMAN NEOCORTICAL SLICES FROM EPILEPTIC PATIENTS E.-J. Speckmann[1*], R. Köhling[1], A. Lücke[1], H. Straub[1], , I. Tuxhorn[2], P. Wolf[2], H. Pannek[3] and F. Oppel[3]
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Human neocortical slice preparations were obtained from patients undergoing surgical treatment of refractory epilepsy. Repetitive sharp field potentials occurred spontaneously, i.e. without external manipulation (cf. Schwartzkroin and Knowles (1984) Science 223: 709-712; McCormick (1989) J. Neurophysiol. 62: 1018-1027), in these preparations. These potentials resembled epileptiform potentials in the EEG (50-300μV, 0.3-0.6Hz) and were associated with sequences of excitatory and inhibitory potentials of surrounding neurons. In this investigation, these potentials were characterized pharmacologically.

The slice preparations (n=30 of 13 patients) were superfused with artificial cerebrospinal fluid. Field potential (FP) and intracellular recordings (n=10) were done from layer III to V. The following agents were added to the superfusate: DL-2-amino-phosphonovalerate (APV, 100 μM, n=11), 6-cyano-7-nitroquinoxalin-2,3-dion (CNQX, 5μM), bicuculline (10 μM), CGP 55845A (10 μM), verapamil (40 μM), phenytoin and carbamazepine (50-100 μM).

FP and postsynaptic potentials were reversibly blocked by the non-NMDA antagonist CNQX, but not by the NMDA antagonist APV. Likewise, the $GABA_A$ antagonist bicuculline reversibly suppressed spontaneous potentials but not the $GABA_B$ antagonist CGP 55845A. The organic calcium channel blocker verapamil, as well as the standard antiepileptic drugs phenytoin and carbamazepine also reversibly blocked all potentials.

These findings suggest that the spontaneous activity is (1) mediated via non-NMDA receptors, (2) possibly synchronized via gabaergic interneurons, (3) generated involving calcium currents and (4) suppressed by antiepileptic drugs.

769.2

ABNORMAL EXCITABILITY OF DENTATE GRANULE CELLS IN HIPPOCAMPAL SLICES FROM TEMPORAL LOBE EPILEPTIC PATIENTS. K.Uruno*, M.J.O'Connor and L.M.Masukawa. Depts. of Neurology and Surgery, University of Pennsylvania Medical School and The Graduate Hospital Research Center, Philadelphia, PA 19146.

We previously reported that hippocampal tissue taken from some temporal lobe epileptic patients during therapeutic lobectomies exhibited field responses that were more excitable than others, and that those excitable responses were significantly correlated with abnormal Timm staining in the molecular layer of the dentate gyrus, neuronal loss in the hilus (Masukawa et al. 1992) or reduced feedback synaptic inhibition or abnormal synaptic inhibition (Uruno et al. 1994, Uruno et al. in press). Our observations are consistent with an imbalance between overall excitation and inhibition. Since those hippocampi with intact or even strong inhibition were taken from patients with refractory seizures, a decrease in feedback inhibition is unlikely the single cause of epilepsy.

In the present study, in addition to field recordings, we used fine electrode intracellular recording from dentate granule cells to examine responses of individual neurons. We injected cells with biocytin or neurobiotin for identification as granule cells. We observed that when field responses showed increased excitability (i.e., multiple population spikes, prolonged field or, biphasic field PSPs, or multiple antidromic population spikes) individual granule cells showed abnormal excitability such as burst firing to a single afferent stimulus with a prolonged EPSP, less or absences of spike adaptation in response to depolarizing current injection, and spontaneous firing/burst. In a group of neurons, slow membrane depolarization with periodic bursts was observed. Our results suggest increased excitability of granule cells, and possibly a change in synaptic circuitry that leads to periodic bursts. (NIH grant # NS23077 to LMM)

769.3

MODULATION OF SYNAPTIC RESPONSES IN RAT AND HUMAN DENTATE GYRUS. Anne Williamson*, Kunio Kato and Dennis D. Spencer, Section of Neurosurgery, Yale University School of Medicine, New Haven, CT 06520

Adenosine is an endogenous transmitter which is released by tonic electrical stimulation. Activation of presynaptic A1 receptors inhibits transmitter release, presumably by decreasing c-AMP or by blocking channels, which reduces neuronal excitability. This presynaptic regulation could be an important factor in epileptogenesis. We have investigated the modulation of synaptic responses by exogenous and endogenous adenosine in rat and human hippocampal slices. Tissue was obtained from patients diagnosed with mesial temporal sclerosis (MTS) or tumor related temporal lobe epilepsy (TTLE). The synaptic reorganization and cell loss seen in MTS are not seen in the TTLE hippocampi.

Application of the A1 agonist cyclohexyladenosine (CHA) decreased the slope of field EPSPs with an ED50 of 0.076 ± 0.120 μM (N=6). In contrast, in slices from MTS and TTLE hippocampi, the ED50s for CHA were much higher (MTS, 0.386 ± 0.685 μM; TTLE 6.64 ± 1.60 μM). In MTS patients, the ED50 appeared to correlate with the degree of sclerosis. We also applied the A1 antagonist DPCPX at 0.2 μM and monitored the slope of the field EPSPs. The slices obtained from MTS patients showed an increase of 45.3± 10.4 % (N=3) while we observed a 32.6 ± 5.1% μM (N=10) in the rat dentate gyrus. These data suggest that there is a lower sensitivity to adenosine in the human dentate gyrus compared to rats. Adenosine may not, therefore, play a major role in presynaptic modulation in the more normal TTLE tissue, but that purinergic mechanisms are present in the MTS tissue.

769.4

DYNORPHIN IMMUNOREACTIVITY IN THE DENTATE GYRUS IN HUMAN TEMPORAL LOBE EPILEPSY: AN ELECTRON MICROSCOPIC STUDY OF REORGANIZED MOSSY FIBER SYNAPSES. N. Zhang* and C.R. Houser. Brain Research Institute, UCLA, and VA Medical Center, Los Angeles, CA 90095

Ultrastructural studies of dynorphin A localization have been conducted to determine the characteristics of presumptive reorganized axon terminals in the dentate gyrus of humans with temporal lobe epilepsy (TLE). Following immersion fixation, surgical TLE specimens were processed for preembedding immunoperoxidase labeling for dynorphin A(1-17). Dynorphin-labeled axon terminals were numerous in the inner molecular layer, and the immunoreactivity was highly associated with dense core vesicles. The labeled terminals varied greatly in size, but many were relatively large (2μm or greater in major diameter), contained high concentrations of clear round vesicles, had irregular shapes, and thus exhibited many of the ultrastructural features of mossy fiber terminals. The majority of dynorphin-labeled terminals formed distinct asymmetric synapses with dendritic spines and small dendrites. The labeled axonal profiles frequently exhibited two or more synaptic contacts with either the same or different postsynaptic elements. Labeled dense core vesicles were also observed near postsynaptic sites of some dendritic spines and shafts. The findings support the suggestion that reorganized dynorphin-containing mossy fiber terminals form abundant functional synapses in inner molecular layer of the dentate gyrus in humans with TLE. Supported by NS21908 and VA Medical Research Funds.

769.5

CHILDHOOD SEIZURES INDUCE HIPPOCAMPAL NEURON LOSSES AND MOSSY FIBER SYNAPTIC REORGANIZATION: GW Mathern *, TL Babb, JK. Pretorius, JP Leite, KM Yeoman, PA Kuhlman, and WJ Peacock. UCLA School of Medicine, Los Angeles, California

This study determined if severe childhood seizures were associated with hippocampal pathology and if hippocampal sclerosis evolved from longer seizure histories. Children with catastrophic epilepsy (n=25) and autopsy (n=23) hippocampal sections were studied for: 1) neuron densities; and 2) gray value density of supragranular neo-Timm's staining. Results showed: 1) Compared to age-matched autopsies, children as young as age 5 years with a history of hippocampal seizures showed markedly decreased granule cell, hilar and regio superior neuron densities in the pattern similar to adult hippocampal sclerosis. By contrast, children with extra-hippocampal pathologies and seizures showed only decreased granule cell densities. 2) The gray value (GV) densities, when compared to autopsies, showed greater supragranular GVs in children with congenital pathologies; however the GVs in children with hippocampal seizures were greatest. 3) Of the children with extra-hippocampal pathologies there were no statistical correlations between longer seizure durations with changes in neuron densities, or mossy fiber sprouting. These results indicate: 1) Extra-hippocampal childhood seizures are associated with moderate fascia dentata and minimal Ammon's horn neuron losses and signs of aberrant mossy fiber sprouting. 2) By contrast, young children with the syndrome of mesial temporal epilepsy show the neuron losses and mossy fiber sprouting typical of hippocampal sclerosis. These findings support the hypothesis that childhood seizures can damage or alter the postnatally developing granule cells of the human hippocampus, and that early neuron losses and aberrant axon circuits may contribute to chronic hippocampal seizures. However, repeated childhood generalized seizures are not necessarily associated with the development of hippocampal sclerosis. Supported by NS 02808, and K08 NS 1603.

769.6

NEUROPATHOLOGICAL FINDINGS IN STATUS EPILEPTICUS. M. G. Hadfield*, C. A. Fortner, R.J. DeLorenzo. Div. Neuropathology, Medical College of Virginia/VCU Richmond, VA 23298.

We correlated post mortem brain lesions with clinical findings in 30 patients with status epilepticus (SE) (27 adults and 3 children between 2 months and 80 years of age). Clinical features: survival time ranged from 0 to 59 days post SE. 70% had no prior seizure history; 50% had acute CNS insults; 20% had acute "non CNS" primary causes for SE; 17% had acute hypoxia or anoxia; 17% had preexisting lesions; and 10% were withdrawing from alcohol or antiepileptic drugs.

Pathologic changes identified: 23% old or recent infarcts and an additional 37% acute anoxic changes; 19% CNS infections; 13% hemorrhages; 7% contusions; 7% tumors; 37% edema; 37% astrocytosis in various parts of the brain, including 10% in the hippocampus; neuronal loss in 37%, primarily in the cerebellum.

To our knowledge, this is the most comprehensive study to date concerned with morbid CNS changes seen in SE. In particular it provides novel data on SE patients with no prior seizure history in an adult population and those with nonconvulsive seizures. The range and extent of post mortem lesions was much greater than clinically suspected.

769.7

Localization of the glutamate receptor subunit GluR1 in the hippocampus of patients with Temporal Lobe Epilepsy. N.C. de Lanerolle*, G. von Campe, M. Brines, I. Kovacs and D.D. Spencer, Section of Neurosurgery, Yale University School of Medicine, New Haven, CT. 06520.

The GluR1 receptor was localized immunocytochemically in hippocampi surgically removed from patients with intractable temporal lobe epilepsy (TLE). mRNA for the flip and flop splice variants of GluR1 were localized by in situ hybridization with specific oligoprobes. Excised hippocampi fall into two broad categories -- those that show sclerosis and reorganization (MTLE) and are seizure foci, and those that do not show reorganization (MaTLE or PTLE) [Clin. Neurosci., 2, 64-84, 1994]. In the **non-reorganized hippocampus** immunoreactivity was weak in the granule cell bodies, but stronger on the apical dendrites throughout the molecular layer (ML). Hilar interneurons were also stained. Within area CA1 to CA3 immunoreactivity was localized to dendrites and not on pyramidal cell bodies. In stratum oriens cell bodies and dendrites were strongly labeled. The flip variant was weakly expressed on granule cell bodies, but strongly expressed on deep hilar neurons, and throughout the pyramidal cell layer of areas CA1 to CA3. The flop variant was only expressed on granule cell bodies. In the **reorganized (MTLE) hippocampus** the pattern of immunoreactivity showed two differences. (1) Surviving hilar neurons appeared to have punctate clusters of immunoreactivity on them. Many of these neurons resemble mossy cells. The same pattern of punctate staining occurred on the cell bodies of some CA3 neurons. These patterns of staining may represent receptor expression on these cell bodies or on mossy fiber terminals that synapse on these cell bodies. (2) In area CA1 where there is loss of pyramidal neurons, small immunoreactive neurons appear scattered in the shrunken pyramidal layer. These cells were not seen without pyramidal cell loss. The relevance of these distinct changes in GluR1 expression in the hippocampal seizure focus remains unclear at present. [Supported by 27081 to N.C. de L]

769.8

MESSENGER RNA AND POSTSYNAPTIC DENSITY PROTEIN ALTERATIONS ARE OBSERVED IN THE EPILEPTIC HUMAN BRAIN.
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Temporal lobectomy specimens obtained from surgeries performed for seizures intractable to anticonvulsants and autopsy specimens from neurologically normal individuals were used for the preparation of synaptosomes and postsynaptic densities (PSD) by a modification of the method described by Cohen et al. Synaptosomes and PSD were obtained from the same specimens. The synaptosomes were analyzed for mRNA using Northern Blot techniques. The presence of 14 mRNA molecules were detected. Among these, the mRNA molecules for brain derived nerve growth factor (BDNF), GAP43 the molecule associated with neuronal growth cones, nerve growth factor (NGF), and tubulin were found to be enriched in the synaptosomes relative to the mRNA for the same proteins in the total temporal lobe. Actin was not enriched in the synaptosomes. When the relative mRNA content (defined as the ratio of the mRNA for the protein in question in the synaptosomes to the mRNA of the protein in the total temporal lobe preparation) in the epileptic brain was compared to the synaptosomes prepared from the control specimens a 2.5 fold increase ($p<0.01$) in BDNF mRNA and a 4 fold increase in GAP43 mRNA ($p<0.001$) was observed in the epileptic brain. In the case of tubulin a statistically significant 2 fold increase was observed in the autopsy specimens when compared to the controls. The NGF and tubulin mRNAs were not significantly different. The analysis by polyacrylamide gel electrophoresis, of the PSD proteins revealed a significant increase in the 20, 30 and 60KD bands of the PSD specific proteins in the epileptic brain. The increase in the GAP43 and BDNF mRNA in the epileptic brain suggests a correlation between the growth factor often observed to be increased in seizures and neuronal sprouting. The alterations in a subclass of the PSD proteins suggests a that a structural alteration of PSD may be associated with repeated seizure activity in the human brain.

769.9

INCREASED TENASCIN-IMMUNOREACTIVITY IN THE HIPPOCAMPUS OF EPILEPSY PATIENTS WITH AMMON'S HORN SCLEROSIS. B. Scheffler[1], H. Beck[3]*, K. Behle[1], A Faissner[2], O.D. Wiestler[1], I. Blümcke[1]. 1) Dept. of Neuropathology, Univ. Bonn Med. Ctr., 53105 Bonn, F.R.G. 2) Dept. Neurobiology, Univ. Heidelberg, 69120 Heidelberg, F.R.G. 3) Dept. Epileptology, Univ. Bonn Med. Ctr., 53105 Bonn, F.R.G.

Tenascin (TN-C) is an extracellular matrix glycoprotein transiently expressed by primarily astrocytes in the developing CNS. Its expression appears to correlate with key events during neurohistogenesis, e.g. neuronal migration and neurite outgrowth. The adult rat hippocampus contains only low amounts of TN-C. Increased TN-C levels have been detected in the lesioned PNS and, to a lower extent, in stabwounds of the CNS. In this study, we have analyzed the distribution of TN-C in 35 surgical hippocampus specimens from patiens with pharmaco-resistant temporal lobe epilepsy, using a panel of anti-human TN-C monoclonal antibodies.

In normal human autopsy specimens, TN-C antibodies preferentially labeled the borders between principal cell layers and layers harbouring fiber tracts, e.g. the alveus or hippocampal fissure. In addition, TN-C immunoreactivity was found within the polymorphic layer of the dentate gyrus. In hippocampal specimens from patients with severe neuronal cell loss (Ammon's horn sclerosis, AHS) a striking increase in TN-C immunoreactivity was observed in all subfields of the hippocampal formation. This increase was pronounced in the dentate gyrus molecular layer, the pyramidal cell layer and the stratum radiatum of the Ammon's horn. The staining pattern reflects the distribution of reactive fibrillary astrocytes in AHS.

An upregulation of TN-C may be indicative of altered glio-neuronal interactions in the hippocampus of epilepsy patients with Ammon's horn sclerosis and contribute to axonal reorganization and sprouting.

769.10

NONLINEAR AUTOREGRESSIVE ANALYSIS OF ICTAL AND INTERICTAL ELECTROCORTICOGRAPHIC RECORDS.
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Electrocorticography (ECoG) permits fine spatial sampling of the electrical fields of the brain. To examine the detailed dynamics of ictal and interictal brain activity, we applied the nonlinear autoregressive (NLAR) fingerprint method (Schiff et al., Biological Cybernetics 1995) to electrocorticographic records obtained from two epilepsy surgery patients with temporal lobe epilepsy. Both patients were chronically implanted with subdural electrodes.

Time series from multiple subdural electrodes were analyzed with a family of NLAR models, each consisting of a single quadratic or bilinear interaction term added to an otherwise linear model. Interictal ECoG segments were best represented by a linear model. Ictal discharges in both patients revealed significant nonlinear signal interactions. Principal components analysis was used to reduce the observed recordings to a minimal set of "generators". This revealed that three or more such "generators" were required. NLAR models of these generators revealed a separate pattern of nonlinear interactions for each generator. In one generator we observed a surprising resemblance to the nonlinear dynamics previously identified in the 3/second spike-wave of petit mal epilepsy, consisting of an interaction of signal values 64 ms in the past with signal values 144 ms in the past. This finding raises the possibility that a common circuit element underlies both types of generalized seizure disorder.

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769.11

NEOCORTICAL AND HIPPOCAMPAL DEFICITS IN TEMPORAL LOBE EPILEPSY L. Marsh, P.K. Shear*, E.V. Sullivan, M.J. Morrell, H. Freeman, A. Marie, K.O. Lim, A. Pfefferbaum Department of Psychiatry and Behavioral Sciences and Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305, and Department of Veterans Affairs Medical Center, Palo Alto, CA 94304

Magnetic resonance imaging (MRI) studies of localization-related epilepsy of temporal lobe origin (TLE) have concentrated on the hippocampus because of the prognostic significance of mesial temporal sclerosis. However, little is known about the extent of volume abnormalities in the neocortex of temporal and extra-temporal brain regions. This MRI study examined whether neocortical volume deficits were present in patients with unilateral TLE, as defined after evaluation for epilepsy surgery. Regions of interest (ROI), measured from 3mm spin-echo coronal images, included the hippocampus as well as gray matter of the temporal lobe, superior temporal gyrus (STG), and a frontal-parietal region (FPR). ROIs were adjusted for normal variation due to head size and age based on 72 controls. Nonparametric tests examined group differences between 7 left-TLE, 7 right-TLE, and an age-matched subsample of 49 controls; all subjects were men. Compared to controls, hippocampal volume deficits were ipsilateral to side of seizure onset in TLE (left-TLE, $p<.002$; right-TLE, $p<.09$). By contrast, relative to controls, both patient groups had smaller gray matter volumes bilaterally in the temporal lobes, STG, and FPR (significant for bilateral FPR and left STG in left-TLE and for bilateral temporal lobe and right FPR in right-TLE). A two-way ANOVA, comparing hippocampal volumes (ipsilateral or contralateral to epilepsy focus) in the two patient groups revealed a significant hemisphere effect ($p<.004$). There were no main effects or interactions in similar ANOVAs for the neocortical ROIs. These data suggest that hippocampal deficits in TLE are related to side of the epileptogenic focus, whereas neocortical tissue volume deficits are bilateral and occur in both temporal and extra-temporal regions.

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769.12

AN EVENT-RELATED POTENTIAL (ERP) STUDY ON DISTURBANCE OF SEMANTIC PROCESSING IN TEMPORAL LOBE EPILEPSY. T. Miyamoto*, J. Katayama[b], Masako Kohsaka, H. Honma, R. Kobayashi, S. Kohsaka[a], H. Okuhara, and T. Koyama Dept. of Psychiat. and Neurol. and [a]Dept. of Pediat., Sch. of Med. and [b]Fac. of Edc., Hokkaido Univ., Sapporo JAPAN

Several lines of evidence have indicated the disturbance of cognitive function, especially memory and language dysfunction in temporal lobe epilepsy (TLE). We focused the semantic memory which is related to semantic processing in language. We investigated the possible disturbance of semantic processing in TLEs with N400, an event-related potential, related to semantic processing.

The seven TLE patients were right-handed and aged 17-38 years. All had complex partial seizures and were taking 1 to 3 kinds of anti-epileptic drugs. The controls were matched for handedness, gender and age.

We used a category matching paradigm consisting of two conditions; the match-condition in which the target word was the category name for the prime word and the mismatch-condition in which the target was of a different category name from the prime. The subjects were required to press one of two buttons accordingly for matches or mismatches.

Reaction times were significantly longer in TLEs than in the controls. The mean amplitudes of N400 in the mismatch-condition in TLEs were smaller than in the controls.

This suggests that there is a disturbance of the semantic processing in TLEs, especially in the mismatch-condition.

769.13

ANTICONVULSANT EFFECTS OF MELATONIN IN HUMANS: TWO CASE STUDIES. TH Champney*, M Sánchez-Forte, A Muñóz-Hoyos, A Molina-Carballo, F Moreno-Madrid and D. Acuña-Castroviejo. Dept Human Anat, Texas A&M Univ, College Station, TX 77843 and Dept Pediat and Dept Fisiol, Univ Granada, Granada, E-18012, Spain.

Melatonin (MEL), a hormone produced by the pineal gland, depresses and/or synchronizes neural activity in numerous species suggesting an anticonvulsive capability. The present case studies describe the effects of MEL administration to two children with untreatable seizures. The first child is a 32 month old female who has had progressive myoclonic epilepsy since one month of age (15 - 20 seizures per day) who was unresponsive to the known anticonvulsants. Ten days after initiating MEL treatment (50 mg, po, at 0900 h and 100 mg at 2100 h) with the anticonvulsants, primidone (125 mg, po, at 0900 h and 250 mg at 2100 h), clonazepam (0.5 mg, po, every 8 h) and phenobarbitone (22.5 mg, po, every 12 h), the seizures disappeared. Presently, she is still receiving the MEL and the anticonvulsants. Clinical tests (EEG, neurologic and psychomotor) all fall within normal ranges and indicate a resumed normal development. The second case involves a 6 year old female with intractable nocturnal seizures that are resistant to the known anticonvulsants. She would experience at least 4 seizures per week shortly after falling to sleep. This produced sleep anxiety and disrupted sleep patterns. After initiating MEL treatment (60 mg daily, po, at 1800 h) and a ketogenic diet, the seizures were reduced to 1 - 2 per week and her sleep pattern improved markedly. She is now receiving 20 - 30 mg of MEL daily which provides good seizure control; reducing the dosage to 10 mg increases seizure production. Both children have been receiving MEL daily for over six months with no apparent adverse side effects. These results suggest that MEL can act as an anticonvulsant in humans, especially in those seizure states that are unresponsive to conventional anticonvulsants. This could be especially important in preventing seizure-dependent neurologic damage in these patients.

769.14

ALUMINA GEL INJECTIONS INTO THE AMYGDALA OF MONKEYS CAUSE BEHAVIORAL AND PATHOLOGICAL CHANGES FOUND IN TEMPORAL LOBE EPILEPSY. C.E. Ribak*, L. Seress, P. Weber, and R.A. E. Bakay. Dept. of Anatomy & Neurobiology, Univ. of Calif., Irvine, CA 92717 and Dept. of Neurosurgery, Emory Univ. Clinic, Atlanta, GA 30322.

When alumina gel is injected into the sensorimotor cortex of monkeys, a seizure focus is created that is similar to that observed in posttraumatic epilepsy in humans. Anatomical studies indicate that the focus displays a preferential loss of GABAergic neurons that may underlie the development of seizures. Alumina gel was injected into the temporal lobes of monkeys to determine whether complex partial seizures could be generated. Timm-stained and electron microscopic preparations were analyzed from these monkeys to determine the types of anatomical changes associated with monkeys with these seizures. Non-injected control monkeys and monkeys with injections into the middle and inferior temporal gyri displayed no seizures and did not have any hippocampal pathology. In contrast, complex partial seizures were observed within 2-3 days in monkeys with injections into the amygdala, entorhinal cortex or hippocampus. Hippocampal pathology was limited to the injection site in monkeys with hippocampal injections and was not observed with entorhinal cortex injections. Monkeys with amygdala injections displayed pathology similar to that described in humans with temporal lobe epilepsy. Thus, Timm staining revealed mossy fiber sprouting into the molecular layer of the dentate gyrus, Nissl staining showed degeneration of neurons in the hilus of the dentate gyrus, CA1 of the hippocampus, and layer III of the entorhinal cortex, and electron microscopic preparations demonstrated the hypertrophy of CA3 pyramidal cell dendrites and the formation of complex spines on the dendrites of granule cells by sprouted mossy fibers. These results describe a non-human primate model of temporal lobe epilepsy that displays similar behavioral and pathological findings as those found in humans.

769.15

A MODEL OF CHRONIC FOCAL EPILEPSY IN SQUIRREL MONKEY

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Introduction: The aim of this study was to develop a model of chronic focal epilepsy, in squirrel monkeys. The model was characterized biochemically and electrophysiologically, using intracerebral microdialysis and electroencephalography (EEG) respectively.

Material and methods. Eight squirrel monkeys were used for the experiments. Chronic focal epilepsy was induced by intracortical injection of 25 µl alumina hydroxid. Intracerebral microdialysis (CMA 12 probes, membrane length 2 mm, flow rate of 2 & 5 µl/min) was performed together with the last EEG recording at the end of the observation period (16-18months) and the samples were analyzed in order to obtain basal levels for various amino acids.

Results: All animals devloped chronic focal epilepsy, persisting during the whole period of observation. The seizures started focally, generalized rapidly and lasted for about 1 minute. Interictally, epileptic spikes were recorded in 7/8 animals 3 months after the intracortical injection. The spikes were restricted to the same side as was operated. After 11 months, the interictal epileptogenic activity could also be recorded contralaterally in three animals and ipsilateral in 4 animals. Basal interictal dialysate levels were obtained in 6 animals. The levels of amino acids were (mean ± SEM [µM]) aspartate: 0.34±0.16, glutamate: 1.32±0.87, serine: 1.00±0.22, glycine: 1.41±0.48, taurine: 0.44±0.08.

Conclusion: Intracortical injection of alumina hydroxid induces chronic focal epilepsy with spontanteous seizures in the squirrel monkey. This model may be used for exploring various treatments of epilepsy, e.g. noninvasive methods as radiosurgery.

770.1

NMDA RECEPTOR BLOCKERS PARADOXICALLY INCREASE SPIKE-WAVE SYNCHRONIZATION AND NEOCORTICAL EXCITABILITY IN A MUTANT MOUSE MODEL OF SPIKE-WAVE EPILEPSY, STARGAZER. W.K. Nahm*, J.L. Noebels. Developmental Neurogenetics Laboratory, Dept. of Neurology, Div. of Neuroscience, Baylor College of Medicine, Houston, TX 77030

The presence of multiple genetic loci expressing spike-wave (SW) activity and the disparity of pharmacological effects on SW discharges in various models suggest the presence of distinct intervening defects subserving SW epileptogenesis. It is believed that SW activity arises when rhythmic firing in a circuit that includes the neocortex, thalamus and reticular nucleus is converted to abnormal oscillations by enhanced hyperpolarizing GABAergic and depolarizing T-type Ca^{2+} channel conductances. It has been hypothesized that NMDA-R activity is required within this circuit, since NMDA-R blockers suppress SW discharges in several models. To determine whether NMDA-R transmission is an essential component of SW seizures in all models, we employed stargazer (*stg/stg*) mutants (Chr. 15) to examine the effects of competitive (CPP) and noncompetitive (MK-801) NMDA-R blockade on in vivo SW activity and on in vitro neocortical 0 Mg^{2+}-induced epileptiform discharges (EDs). At doses that suppress SW activity in other animal models, CPP (40-60μM/kg) and MK-801 (1.6-2.0μM/kg) paradoxically initiate nearly continuous SW activity in *stg/stg* mice, while they do not affect EEG activity in wild type (+/+) mice. In field recordings from cortical slices bathed in 0 Mg^{2+} aCSF, CPP (10μM) and MK-801 (1-10μM) dramatically increase the frequency [(10μM CPP, 311%) (1μM MK-801, 310%; 3μM MK-801, 375%; 10μM MK-801, 431%)] and decrease the duration [(10μM CPP, 735%) (1μM MK-801, 210%; 3μM MK-801, 424%; 10μM MK-801, 979%)] of spontaneous EDs recorded in layer 4/5 of *stg/stg* slices; while they abolish or greatly attenuate both these parameters in +/+ slices. These data suggest that NMDA-R activation is not an obligatory step in the final pathway of burst generation in all models of SW epilepsy, and point to a specific excitability defect linked to NMDA-R-mediated transmission in *stg/stg* neocortical cells.

770.2

"*slow-wave epilepsy*", A MUTANT MOUSE WITH 3/SEC SPIKE-WAVE SEIZURES. Jeffrey L. Noebels[1]*, Cathleen M. Lutz[2], Audrey Fu[2], Wayne N. Frankel[2]. [1]Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030 and [2]Jackson Laboratory, Bar Harbor, ME 04609.

Electrocorticographic survey of murine neurological mutations has so far revealed 5 recessive loci displaying spontaneous 6-7/sec spike-wave discharges accompanied by behavioral arrest. The seizures are selectively suppressed by ethosuximide, and provide useful gene models of the aberrant thalamocortical oscillations in inherited human generalized absence epilepsies. Here we report a new mutant with a novel epilepsy phenotype that exhibits frequent spike-wave seizures in the 3/sec range. The recessive *swe* mutation arose on the SJL/J inbred background and maps to the distal portion of chr. 4 between D4Nds2 and D4Mit42. Chronic ECoG recordings reveal very frequent (~118/hour) episodes of generalized, bilaterally symmetric wave, and wave-spike activity. The rhythmic periodicity ranges from 3-4.5/sec. Individual durations range from 1-68 secs (mean 3.5 ± 0.7 SEM sec). The seizures are triggered by waves; in some longer discharges, the spike component is absent or buried in the wave. Both the shorter wave-spike bursts and longer wave patterns are associated with behavioral arrest, and are rapidly abolished by ethosuximide (50-100 mg/kg). No 6-7/sec or higher discharges are present. On this inbred background, rare spontaneous generalized tonic-clonic seizure episodes have also been observed in *swe* homozygotes as early as 14-18 days postnatal. By 12-14 days, the mutants develop locomotor ataxia, but no other obvious neurological deficits are evident. The *swe* mutant demonstrates that specific cortical oscillation frequencies are linked to the function of distinct genes, and provides a critical entry point into the molecular dissection of mechanisms regulating spike-wave epileptogenesis.

770.3

CORTICAL HYPEREXCITABILITY IN THE SPIKE-WAVE EPILEPTIC MUTANT MOUSE *STARGAZER*. Eric Di Pasquale, Karl D. Keegan* and Jeffrey L. Noebels, Dept. of Neurology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

Inherited spike-wave epilepsy arises from aberrant bursting in thalamocortical circuits, but the site of gene-linked hyperexcitability is not known. Recordings were performed in brain slices of adult *stargazer* mice (*stg/stg*) and their coisogenic controls (+/+). Field recordings in thalamocortical slices bathed in aCSF revealed the presence of spontaneous low frequency, synchronous network discharges in all layers of *stg*, but not +/+ neocortex. The mutant discharges were not abolished by section of the thalamocortical projection fibers. Intracellular and whole-cell recordings in cortical layer IV-V *stg* neurons showed spontaneous giant depolarizing epsps generating bursts of action potentials with little after burst hyperpolarization. The after burst hyperpolarization seen in +/+ neurons during bursting induced by 0 Mg^{2+} saline was almost absent in *stg* (6.9±1.1 mV vs 1.5±0.5 mV respectively).

In whole-cell configuration, two classes of regular-spiking neurons were identified in similar ratios in both genotypes: RS1 (+/+ 16/20; *stg* 17/22), and RS2 (+/+ 4/20; *stg* 5/22). *Stg* single action potential (AP) half width, rise time and decay time were significantly decreased by 12%, 15% and 13% respectively. The rheobase intensity was significantly decreased by 58% in *stg* (from 80 pA to 25 pA). No differences were observed in single AP overshoot and AHP, afterburst hyperpolarization, resting potential, input resistance, or time constant. The *f-I* slope was significantly increased by 29% in *stg* (*stg* 217.7 Hz/nA; +/+ 154.4 Hz/nA). Anomalous rectification (AR) was observed in both genotypes, but a depolarizing "sag" was strongly enhanced in *stg* (8.6 ±1.2 mV) relative to +/+ (1.6±0.6 mV). These results demonstrate an increase in network and intrinsic excitability in *stargazer* cortical neurons that could lower the threshold for thalamocortical oscillations in this model of inherited spike-wave epilepsy. Supported by NIH NS29709 and PHILIPPE Foundation.

770.4

AUDIOGENIC SEIZURES INDUCE EXPRESSION OF FOS IN THE NUCLEUS LOCUS COERULEUS OF GENETICALLY EPILEPSY PRONE RATS. R.W. Clough*[1], J.B. Eells[1], P.C. Jobe[2] and R.A. Browning[1]. [1]Sch. of Med., Southern Illinois Univ., Carbondale, IL 62901 and [2]Sch. of Med., Univ. of Illinois, Peoria, IL 61656.

Genetically epilepsy-prone rats (GEPRs) have an innate and widespread deficiency in the noradrenergic neurotransmitter system which contributes to their propensity for seizures in response to audiogenic stimuli. GEPRs of the mild-seizure substrain (GEPR-3s) display enhancement of seizure severity following depletion of norepinephrine (NE) using 6-hydroxydopamine while augmentation of noradrenergic function in the GEPR brain has an antiepileptic effect in the GEPR. Since epileptic propensity in these animals involves NE hypofunction which may result from failure of activation of NE perikarya, the present study sought to discern whether the nucleus Locus Coeruleus (LC), a major noradrenergic nucleus, exhibits activation of the immediate-early-gene Fos coincident with seizures. Seizures were induced using a 100 db signal. 2.5 hours following seizures, rats were transcardially perfused and prepared for immunohistochemical processing of brain sections for Fos. Quantifiable morphometric results demonstrated a profound activation of Fos immunoreactivity in neurons of the LC subsequent to seizure. These findings suggest that the LC perikarya are highly activated during audiogenic seizures, and perhaps, that deficiencies in other components of the NE system (ie, terminals) may be responsible for the lack of seizure suppression in the GEPR. Supported by Bayer Pharmaceuticals and SIU.

770.5

COINCIDENT MATURATION OF AUDIOGENIC SEIZURE PROPENSITY AND FOS ACTIVATION PATTERNS IN DEVELOPING GENETICALLY EPILEPSY PRONE RATS. J.B. Eells*[1], R.W. Clough[1], P.C. Jobe[2] and R.A. Browning[1]. [1]Sch. of Med., Southern Illinois Univ., Carbondale, IL 62901 and [2]Sch. of Med., Univ. of Illinois, Peoria, IL 61656.

The mild seizure substrain of Genetically epilepsy-prone rats (GEPR-3s) exhibit an interesting developmental pattern of audiogenic seizure (AGS). At 16 days of age, they exhibit a brief run without a seizure in response to an auditory stimulus . At 21 days of age, a brainstem seizure score 3 is followed by facial and forelimb clonus. Seizure severity is increased at 23 days of age to seizure score 9, afterwhich seizure severity decreases again to score 3 by 45 days of age. This study examined possible differences in Fos activation patterns associated with expression of variant seizure types across development in GEPR-3s. Brain sections from rats of 16, 21, 23, and 45 days of age were processed for Fos immunohistochemistry 2.5 hours after AGS. 16 day old GEPR-3s did not exhibit AGS; however, they showed dense Fos immunoreactive (FI) neurons in the pontine nuclei, supraoptic nucleus and the parietal cortex with some Fos labeling in the inferior colliculus and superior olivary nucleus. At 21, 23, and 45 days, rats showed a similar pattern of Fos distribution after AGS; however, seizure severity differed. FI neurons were found in the peripeduncular area, central gray, cuneiform nucleus, paralemniscal area, locus coeruleus and gigantocellular reticular nucleus. Although seizure severity differed, no differences in Fos immunolabeling was observed with different seizure types. Fos immuohistochemistry suggests that these seizure types share a similar neuroanatomical circuitry. Supported by Bayer Pharmaceuticals and SIU.

770.6

ENHANCEMENT OF THE ANTICONVULSANT EFFECTS OF FLUOXETINE BY BLOCKADE OF THE SEROTONIN 5-HT_{1A} RECEPTOR IN GENETICALLY EPILEPSY-PRONE RATS (GEPRs). R. A. Browning[1]*, A. V. Wood[1], M. A. Merrill[1], J.W. Dailey[2] and P.C. Jobe[2]. [1]Dept. Physiol. Southern Illinois Univ, Sch. Med. Carbondale, IL 62901 and [2]Dept. Basic Sciences Univ. Illinois Coll. Med. Peoria IL 61656.

GEPRs are known to have a deficiency in brain serotonin (5-HT). Moreover, pharmacological treatments that increase 5-HT in the synaptic cleft, such as fluoxetine, have been shown to protect these animals against sound-induced (audiogenic) seizures, while treatments that lower the concentration of 5-HT have proconvulsant effects. Inasmuch as recent evidence shows that 5-HT_{1A} antagonists markedly enhance the increase in extracellular 5-HT produced by 5-HT reuptake inhibitors, it was of interest to determine whether such drugs also potentiate the anticonvulsant action of the 5-HT reuptake inhibitor, fluoxetine (FLU), in GEPRs. In the present study female severe-seizure GEPRs (GEPR-9s) received FLU 2 hours before seizure testing and the 5-HT_{1A} antagonist either 30 min or 60 min before testing. The audiogenic seizure response score (ARS), a measure of seizure severity, was significantly ($P<0.05$) reduced when a dose of FLU (15 mg/kg), which alone had no effect on the ARS, was combined with one of the following 5-HT_{1A} antagonists: pindolol (10 mg/kg; ARS reduced from 9 to 4.7); (±) LY 206130 (5 mg/kg, ARS reduced from 9 to 3.9); (-) LY 206130 (2.5 mg/kg, ARS reduced from 9 to 6.0). The mean ARS in GEPR-9s treated with saline or a 5-HT_{1A} inhibitor alone was 9.0, while rats treated with FLU alone had a mean ARS of 8.4 - 9.0. The effect produced by (-) LY 206130 was dose-dependent. Moreover, the anticonvulsant action of FLU in combination with LY 206130 was shown to depend on 5-HT, since it was absent in animals depleted of brain 5-HT by pretreatment with p-chlorophenylalanine ethyl ester (150 mg/kg/day for 3 days). These findings provide further evidence that the anticonvulsant effects of FLU are mediated through 5-HT and that the increase in extracellular 5-HT observed following a 5-HT reuptake inhibitor in combination with a 5-HT_{1A} antagonist is functionally relevant.

770.7

ANTICONVULSANT EFFECT OF ENHANCEMENT OF NORADRENERGIC TRANSMISSION IN THE SUPERIOR COLLICULUS IN GENETICALLY EPILEPSY-PRONE RATS (GEPRS): A MICROINJECTION STUDY. Q.S. Yan*, J. Dickerson, P.C. Jobe and J.W. Dailey. University of Illinois College of Medicine, Peoria, IL. 61656.

An expanding body of data has demonstrated that the seizure prone state in genetically epilepsy-prone rats (GEPRs) is partially caused by deficits in central nervous system noradrenergic transmission. The purpose of this work was to trace etiologically significant noradrenergic terminals to one specific brain area, the superior colliculus (SC). Several drugs with different mechanisms of enhancing noradrenergic transmission were chosen to study. Guide cannulae were implanted just above the SC of female severe seizure GEPRs (GEPR-9s) under anesthesia. Five to seven days later, injection cannulae were bilaterally inserted into the SC while the animal was awake. The drugs or vehicle were administered at a rate of 0.2 μl/min over 5 min. The rats were tested for audiogenic seizure intensity at 0.25, 0.5, 1, 2, and 3 hrs after injection. Bilateral injection of vehicle or prazosin (1 μg/side), or unilateral injection of nisoxetine (8 μg), produced no reduction in the intensity of the audiogenic seizure. Desipramine (2, 4, 8 μg/side), nisoxetine (2, 4, 8 μg/side), and idazoxan (0.25, 1, 4 μg/side) all decreased the seizure intensity in a dose-dependent fashion. Significant decreases in the seizure intensity were also observed after administration of both methoxamine (0.15 μg/side). and phenylephrine (0.15 μg/side). Pretreatment with prazosin (1 μg/side) significantly decreased the anticonvulsant effectiveness of methoxamine (0.15 μg/side) and nisoxetine (8 μg/side). These results suggest that noradrenergic transmission in the SC may be involved in the seizure regulation in the GEPR model, and that this regulation may be mediated, at least in part, by α_1 - receptors. (Supported in part by UICOM-P IRG-1994 to QSY.)

770.8

NOREPINEPHRINE AND SEROTONIN RELEASE DURING SEIZURE IN GEPRs AND NON-EPILEPTIC RATS: ANALYSIS OF ONE MINUTE MICRODIALYSIS SAMPLES **P.C. Jobe, V.U. Deoskar, R.L. Burger, J.W. Dailey* K.H. Ko[1] and P.K. Mishra Department of Basic Sciences, Univ. of Illinois College of Medicine, Peoria, IL 61656 and [1]Seoul National University, Seoul, South Korea.**

Forebrain and brainstem seizure predisposition in GEPRs appears to stem partially from noradrenergic deficits in the superior colliculus and ventrally adjacent structures. Accordingly, seizures induced by stimuli such as electroshock are thought to be more severe in GEPRs than in non-epileptic animals partly because of a reduced capacity of the epileptic animals to release norepinephrine (NE) and/or serotonin (5-HT) in response to seizure discharges. The current work was designed to compare seizure-induced release of monoamines in the terminal fields of the superior colliculus of non-epileptic rats with that of the moderate seizure GEPR (GEPR-3).

All-fused silica microdialysis probes (3 mm, loop-type) were inserted in preimplanted guides in the superior colliculus region of GEPR-3s and non-epileptic control animals. Two hours after the probe insertion, three one-min baseline samples were collected. Maximal electroshock seizures (MES) were induced after collecting four more post handling samples. Four additional one-min samples were collected following MES. These one-min microdialysates were analyzed using a high sensitivity microbore-microflow HPLC-EC system (Mishra, et al., Soc. Neurosci. Abstr., Vol. 21). Following seizure induction, a 6-12 fold increase in extracellular NE and 4-9 fold increase in extracellular 5-HT was observed in normal animals. In contrast, in GEPR-3s the release of NE and 5-HT was limited to only 2-4 fold. The highest extracellular concentrations were observed in the one-minute sample collected immediately following the seizure. The levels returned to near baseline levels within 10 minutes. These observations extend earlier indirect evidence by providing direct documentation for seizure-induced increments in NE and 5-HT release. Also, the experiment provides the first direct evidence that deficits in NE and 5-HT levels in the extracellular fluid characterize ictal episodes in GEPRs. (Supported in part by NS32628).

770.9

DIFFERENTIAL SENSITIVITY TO COCAINE-INDUCED SEIZURES IN TWO STRAINS OF THE GENETICALLY EPILEPSY-PRONE RAT (GEPR). C.E.Reigel*, H.Whitehead, A.T.Lovering and B.-K.Lin. Dept. of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Cocaine is a local anesthetic that also blocks the uptake of dopamine (DA), norepinephrine (NE) and serotonin (5-HT). Because local anesthetics induce seizures in high doses, cocaine has long been believed to induce seizures through its local anesthetic effects. We determined the potency of cocaine, bupropion (DA uptake inhibitor), imipramine (NE/5-HT uptake inhibitor) and procaine (similar local anesthetic potency to cocaine) in producing seizures in 3 rat strains. Intravenous seizures thresholds were determined in GEPR-9s (severe seizure strain), GEPR-3s (moderate seizure strain) or Sprague-Dawley (SD) rats, the strain from which the GEPR was derived.

Behaviorally, seizures produced by all 4 drugs were similar. The 4 drugs produced a similar rank order of potency in all 3 strains. Cocaine was the most potent convulsant, followed by bupropion. Imipramine demonstrated intermediate potency. Procaine was by far the least potent in producing seizures. Cocaine seizure threshold was lower in GEPR-9s than SD controls. Imipramine and procaine seizure thresholds were also lower in GEPR-9s. Cocaine seizure threshold was elevated in GEPR-3s over SD controls. Bupropion, imipramine and procaine seizure thresholds were also elevated in GEPR-3s, with the greatest elevation in bupropion seizure threshold (74 percent over SD controls). The resistance of GEPR-3s to the convulsant effects of cocaine as compared to GEPR-9s and SD controls may reflect differences at the level of the DA transporter. The rank order of potency demonstrated by these 4 drugs supports a dopaminergic involvement in cocaine-induced seizures. (Supported by NIH NS 28118.)

770.10

EXPRESSION OF NOREPINEPHRINE AND DOPAMINE TRANSPORTER MRNA AND TYROSINE HYDROXYLASE MRNA IN THE GENETICALLY EPILEPSY-PRONE RAT P. Szot*, C.E. Reigel and R.C. Veith GRECC, Seattle VAMC, WA 98108 and Dept. of Psychiatry and Behavioral Sciences, Univ. Washington, Seattle, WA 98195 and Dept. of Pharmacology, Texas Tech Univ., Lubbock, TX 79430.

The genetically epilepsy-prone rat (GEPR) is composed of two independent models of epilepsy; the GEPR-3 which exhibit mild generalized clonus and the GEPR-9 which exhibit more severe tonic extensor convulsions in response to acoustic stimuli. The most striking alteration in neurotransmitter function in these animals occurs in the noradrenergic and sertoninergic systems. The dopaminergic system appears to remain unaltered in both models, however the GEPR-3's have an elevated seizure threshold to cocaine (dopamine re-uptake inhibitor) (Reigel et al. 1995, NS Absts). Since the synaptic level of these neurotransmitters are regulated by specific re-uptake proteins and rate-limiting enzymes, the mRNA expression of these proteins was determined in both GEPR models which were seizure naive, as well as controls using in situ hybridization. Expression of norepinephrine transporter (NET) and tyrosine hydroxylase (TH) mRNA was determined in the locus coeruleus (LC) the major noradrenergic locus. NET mRNA expression was significantly elevated in the GEPR-3's compared to control. GEPR-9 NET mRNA expression was not significantly different compared to control or GEPR-3. However, TH mRNA expression in the LC in GEPR-9 was significantly reduced compared to GEPR-3, but not to control. The expression of dopamine transporter (DAT) and TH mRNA was determined in the ventral tegmentum/substantia nigra compacta region. DAT mRNA expression was significantly reduced in GEPR-3's compared to control and GEPR-9's. Unlike the noradrenergic neurons, TH mRNA expression in the dopaminergic neurons was significantly elevated in both GEPR-3 and GEPR-9's. These data indicate that there are regulatory changes in both the noradrenergic and dopaminergic systems of GEPR-3's and 9's and that the GEPR-3 model of epilepsy demonstrates the greatest changes in both NET and DAT mRNA expression. (Supported by Veterans Affairs).

770.11

A QUANTITATIVE COMPARISON OF INHIBITORY SYNAPTIC CURRENTS IN THE HIPPOCAMPUS OF THE LH/LH MOUSE MODEL OF GENERALIZED ABSENCE EPILEPSY. S. J. Caddick* and D. A. Hosford. Neurology Res.,Duke & Durham VA Med.Centers,VAMC Bldg 16, Durham NC 27705.

Absence seizures represent the synchronized burst firing (spike wave discharges) of populations of cortical and thalamic neurons in an oscillatory fashion. Previous work in this laboratory has suggested an upregulation of postsynaptic $GABA_B$ receptor function in the hippocampus of lethargic (lh/lh) mice, a genetic model of absence seizures[1]. In order to look directly at inhibitory transmission, whole cell recordings of locally evoked synaptic currents were obtained from CA1 pyramidal cells in coronal slices (450μm). The peak amplitude of the inhibitory GABAergic currents mediated by $GABA_A$ ($IPSC_A$) and $GABA_B$ ($IPSC_B$) receptors were compared between lh/lh & non-epileptogenic littermates (+/+). IPSC's were isolated using DNQX (20μM) and DL-APV(20μM) and bicuculline (10μM)to isolate $IPSC_B$. We observed no significant differences in the peak amplitude and latency to peak of $IPSC_A$ (lh/lh, 286±41pA, 12±2ms; +/+, 211±28pA, 14±1ms; *P> 0.05*); peak amplitude and latency to peak of $IPSC_B$ (lh/lh, 42±7pA, 148±6ms; +/+, 42±3pA, 161±6ms; *P> 0.05*). These data indicate that there are no changes in the amplitude of the *poststynaptic* IPSC's in pyramidal cells of CA1 taken from lh/lh mice. Further work on comparing the presynaptic $GABA_B$ inhibition of IPSC's and EPSC's is at present underway.

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1. Hosford et al, *Science* 257:398.

770.12

INVOLVEMENT OF THE INDIRECT STRIATO-NIGRAL PATHWAY IN THE CONTROL OF EPILEPTIC SEIZURES. C. Deransart, C. Marescaux and A. Depaulis*, INSERM U. 398, Faculté de Médecine, 11 Rue Humann, 67085 Strasbourg cedex, France.

In order to investigate the involvement of nigral excitatory amino acid transmission in the control of generalized seizures, drugs were bilaterally microinjected in the substantia nigra pars reticulata in two genetic models of epilepsy in the rat. The effects of blockade of the nigral glutamatergic input from the subthalamic nucleus were then examined.

In Wistar rats with spontaneous absence, intranigral injections of dizocilpine, CGP 40116, or 5,7-dichlorokynurenic acid, respectively non competitive and competitive NMDA receptor antagonists and glycine NMDA-associated site antagonist, significantly suppressed spike-and-wave discharges in a time and dose-dependent way (600 and 800; 2 and 4; 500 and 1,000 pmole/side respectively). By contrast, no significant suppressions were observed after intranigral injections of antagonists acting at other glutamatergic receptors. Furthermore, bilateral injections of muscimol (17.5 and 35 pmoles/side) in the subthalamic nucleus suppressed absence seizures in the rat.

In a model of convulsive seizures (audiogenic seizure), bilateral intranigral injections of CGP 40116 (20 and 40 pmoles/side), resulted in a suppression of tonic seizures. No suppressions were observed after bilateral microinjections in the subthalamic nucleus of muscimol at doses up to 700 pmoles/side.

These results show that blockade of NMDA receptors within the substantia nigra pars reticulata suppresses both generalized convulsive and non-convulsive seizures in the rat. Furthermore, the suppression of absence seizures after inhibition of subthalamic neurons suggests the involvement of the indirect striato-nigral pathway in the triggering of the nigral control of epilepsies.

770.13

NMDA Antagonists and Glutamate Release-Inhibitors Suppress Seizures in the DBA/2 Mouse. D. Berlove*, O. Amitay, S. Goldin, L. Margolin and W. Holt. Pharmacology Department, Cambridge NeuroScience, Cambridge, MA 02139.

The DBA/2 mouse is known for its extreme susceptibility to audiogenic seizures. These seizures are triggered by intense, high frequency sound and follow a stereotypic course, beginning with a wild running phase that may rapidly progress to clonic seizures, tonic extension and death. Seizures appear to be the result of an imbalance between excitatory and inhibitory transmission in DBA/2 mice. Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system. Thus a block of glutamate release or receptor binding may prevent or attenuate seizure activity in these mice. We tested the efficacy of the noncompetitive NMDA antagonists MK-801 and CNS 1102 in this assay. Both are highly effective at suppressing audiogenic seizure activity; MK-801 at a dose of 0.25 mg/kg, ip and CNS 1102 at 2.0 mg/kg, ip. The relative *in vivo* potencies of these compounds closely matches their relative *in vitro* potencies at the NMDA receptor, suggesting that blockade at this receptor is responsible for seizure suppression. For pretreatment times up to 4 hours, CNS 1102 significantly reduced seizure severity; no morbidity or mortality was associated with CNS 1102 treatment. We then examined 4 compounds that are believed to block the synaptic release of glutamate: lamotrigine, riluzole, BW619C89, and CNS 1237. All were effective at reducing seizure incidence and severity, although at higher doses than were required for NMDA antagonists. The DBA/2 mouse audiogenic seizure model is a rapid and highly reproducible method of assessing a compound's ability to block glutamatergic neurotransmission. The ability of both NMDA antagonists and inhibitors of glutamate release to prevent audiogenic seizures suggests these seizures may be glutamate-mediated. Drugs selected with this model may have therapeutic value not only for the treatment of epilepsy, but also for other disorders associated with glutamate excitotoxicity including stroke and traumatic brain injury.

770.14

LAMOTRIGINE DRAMATICALLY DECREASES CONTENT OF DOPAMINE METABOLITES IN CAUDATE NUCLEUS OF NORMAL AND SEIZURE-PRONE BALB/C MICE. J.P. Vriend* and N.A.M. Alexiuk. Department of Anatomy, University of Manitoba, Winnipeg, Canada R3E 0W3.

Lamotrigine (LTG) is a relatively new anticonvulsive agent. Little is known concerning its neurochemical mechanism of action. In our laboratory we tested LTG for its anticonvulsive effects in an audiogenic seizure-prone strain of Balb/c mice. The initial part of this project was designed to study the neurochemical effects of LTG at clinically relevant doses. A report of its use in Parkinson's disease (Zipp et al., 1993) led us to examine the effects of LTG administration on the content of dopamine (DA) and its metabolites in the striatum. LTG administered ip, 40 minutes prior to bell testing, inhibited tonic-clonic audiogenic seizures in a dose-dependent fashion. Complete inhibition of seizures was observed at doses between 10 and 20 mg/kg. At these doses, LTG had no significant effect on DA content in extracts of caudate nucleus micropunches. However, LTG administration significantly reduced the content of the DA metabolites, DOPAC and HVA, in caudate nucleus of both seizure-prone and seizure-resistant mice. DOPAC concentrations were reduced to 43% ($p<0.001$) of controls in seizure-resistant mice, while a reduction to 34% ($p<0.001$) of controls was observed in the seizure-prone strain. HVA concentrations were reduced to 81% ($p<0.05$) in seizure-resistant mice, while a reduction to 58% ($p<0.01$) was observed in seizure-prone mice. While these data do not provide evidence for a role of DA in the anticonvulsant action of LTG, the dramatic effects of this drug on the content of DA metabolites suggest that LTG is an inhibitor of DA turnover. An alternative explanation is that LTG reduces intracellular levels of metabolites by inhibiting reuptake of DA and its subsequent metabolism to DOPAC and HVA. A question that arises from this work is whether the inhibitory action on DA metabolite content is secondary to an action on glutaminergic or GABAergic neurons.

770.15

MIDBRAIN 3D DIPOLE MAPPING OF ACOUSTIC EVOKED POTENTIALS IN AUDIOGENIC SEIZURE RESISTANT ANESTHETIZED RATS. Moraes, M.F.D.*, Barboza, A. Del Vecchio, F. and Garcia-Cairasco, N. Neurophysiology and Experimental Neuroethology Laboratory, Physiology Department, Ribeirão Preto School of Medicine. University of São Paulo, Ribeirão Preto, 14049-900. S. P, Brazil.

The inferior colliculus (IC) is the most critical structure of the auditory midbrain involved in audiogenic seizures (AS), a model of experimental epilepsy. AS are induced in susceptible (S) rats, in contrast to resistant (R) rats, by high intensity acoustic stimulation. Thus, in order to disclose functional differences in S and R rats, we began mapping wave form patterns of midbrain acoustic evoked potentials (AEPs) in R Wistar rats. Data were collected from anesthetized rats (thionembutal 40 mg/kg; 240-280 g b.w), by means of carbon fiber electrodes(Tip=10μm; R=35kohms, developed in our laboratory) in the following coordinates, at 0.5mm intervals: AP(lambda) from +0.5 to -2.0mm; L(midline) from 0.5 to 3.0mm; V(dura mater) from 1.5 to 7.0mm; therefore, the mapping procedure resulted in 360 recorded sites (n=3 per site). Monaural pure tone bursts of 3 kHz (90dB) were applied to the contralateral ear. The collected AEPs were promediated (n=50) by means of an A/D converter interfaced with a computer(BIOPAC) and the data expressed as a percentage of maximum of each lateral sweep. Small electrolytic lesions labeled recording points. The results of AEP mapping are presented as conventional 20 ms recordings and used to calculate instantaneous electrical dipoles in each 0.5mm^3 control volume. The calculated dipoles enabled us to reconstruct a cube of the mesencephalon in which the IC is inserted. Results of 3D reconstruction from 0 to 20 ms on a 1ms time interval suggest the signal coming in at 3 ms through the lateral lemniscus, 5 ms in the IC, in 6 ms the signal splits to the superior colliculus, external and dorsal IC nuclei and also projects to the contralateral IC. At around 8ms, central IC seems to receive feedback information from external nuclei. After 9 ms, no significant dipoles are recorded in the cube. Ongoing studies are highlighting differences and similarities between the dynamic (time dependent) 3D dipole distribution of S and R rats.

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770.16

EFFECT OF NITRIC OXIDE SYNTHASE INHIBITION IN DIFFERENT EPILEPTIC SEIZURE MODELS. Del Bel E.A., Oliveira P.R., Oliveira J.A.C., Mishra P.K[1]., Jobe P.C.[1], Garcia-Cairasco, N*. Physiology Department, Ribeirão Preto School of Medicine. University of São Paulo, Ribeirão Preto, Brazil. [1]Department of Basic Sciences, College of Medicine at Peoria, University of Illinois, Illinois, USA.

Discrepant results have been found concerning the role of nitric oxide (NO) in epileptic seizures. Inhibition of endogenous NO production is an adequate tool for establishing a role for NO in brain excitability. We evaluated the effect of a NO synthase (NOS) inhibitor (L-NOARG), 25 mg/kg, twice a day, 4 days and 125 mg/kg (i.p.; 30 min before seizure or drug injection), in pilocarpine, pentylenetetrazol (PTZ) and audiogenic seizures (AS) on male Wistar resistant (R) rats or genetic epilepsy-prone rats (GEPR-3s) and Sprague-Dawley controls (SD).

Eighty eight percent of the rats (X^2; $p<0.05$) had seizures elicited by a sub-convulsant dose of pilocarpine (100 mg/kg, i.p., 1 mg/kg methyl scopolamine, n=8 per group), only after NOS inhibition. NOS inhibition had no effect on sub-convulsant doses of PTZ (15 and 30 mg/kg i.p., n=8), but potentiated the severity of limbic seizures induced by PTZ (60 mg/kg i.p.; n=16). However, the L-NOARG treatment protected against tonic seizures and lethality induced by PTZ (80 mg/kg i.p.; n=8). L-NOARG treatment had no effect on eliciting AS (n=8 per group) in Wistar R rats either 30 min or 24 h after L-NOARG injection. Moreover, GEPR-3s and SD did not modify their severity indexes or seizure latencies after similar treatments. In conclusion, these results suggest that the effect of NOS inhibition on seizure activity depends on the seizure inducing agent and in the case of chemical induction (i.e. PTZ) it depends also on the dose.

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770.17

PROPERTIES OF DEVELOPING RAT HIPPOCAMPUS CA1 NEURONS IN THE GENETICALLY EPILEPSY PRONE RATS (GEPRs) S. Verma-Ahuja, T. L. Pencek* and M.S. Evans, Department of Surgery, Division of Neurosurgery and Department of Neurology, Southern Illinois University, School of Medicine, Springfield, Illinois 62704

The developing GEPRs like normal young rats show a greater propensity for tonic seizure induction during the first two weeks of postnatal development. In the third and fourth weeks, the GEPRs lack the developmental decrease in tonic seizure susceptibility that occurs in normal rats. The adult GEPR hippocampus shows an increased excitability in the CA1 region with paired pulse stimulation, a decreased $GABA_A$ mediated inhibition, reduced spike frequency adaptation with a reduced slow afterhyperpolarization (AHP) in some neurons. We now studied the properties of developing hippocampal CA1 neurons in GEPRs (n=32) and SD rats (n=9). The membrane input resistance was higher in the developing CA1 neurons in GEPRs. The fast AHP was absent in GEPR and the repolarization was slower. The slow AHP was not significantly different in developing GEPR CA1 neurons. All the cells studied in the CA1 region in the SD rats during the first three weeks showed a spike frequency adaptation. In 40% of the GEPR CA1 cells recorded in the 3rd or 4th week of development, the spike frequency adaptation was absent. On single synaptic stimulation, some of the CA1 cells showed burst of action potentials in the 3rd week. Decreased inhibition with repetitive stimulation was seen in developing GEPR rats. Single synaptic stimulation in the presence of 10 μM bicuculline elicited a burst of action potentials lasting about 30 ms in SD rats at three weeks. In GEPRs, however, a prolonged discharge lasting for upto 250 ms was seen in CA1 neurons. These results show an increased excitability with epileptogenic properties in the GEPR CA1 neurons at the time when behavioral seizures are beginning to appear.

771.1

GABA-LIKE IMMUNOREACTIVITY (GABA-LI) IN MOSSY FIBERS OF HIPPOCAMPAL BRAIN SLICES. K.S. Wilcox*, M. Price, B. Johnson, R. Sloviter[2], & M.A. Dichter. Depts. of Neurology and Pharmacology, University of Pennsylvania, Philadelphia, PA, 19104 and [2]Helen Hayes Hospital, W. Haverstraw, NY 10993

Dentate granule cells provide the main excitatory input to the CA3 pyramidal cells of the hippocampus and are thought to utilize glutamate as a transmitter. However, recent work (see Sloviter *et al.*, this meeting) demonstrates that dentate granule cells normally contain both GAD-67 and GABA-LI. When perforant path stimulation is used to induce seizure activity, granule cell GAD and GABA staining increases selectively. We were interested in reproducing this phenomenon in the acute hippocampal slice. Rat hippocampal vibratome slices (400-500 μM) were placed in oxygenated Ringer solution at 32-34° for varying lengths of time. Some slices were incubated in Ringer solution lacking Mg^{2+} to induce seizure-like activity, while others were placed in either standard Ringer or Ringer containing CNQX and APV to block excitatory neurotransmission. Following incubation, sections were fixed with 4% paraformaldehyde and cryoprotected by sucrose. Brain sections (10 μm) were stained with standard protocols for immunoreactivity with a monoclonal GABA antibody (Sigma). Slices that were fixed immediately after dissection showed GABA-LI in the mossy fiber terminals, while brain slices incubated under all other conditions demonstrated more intense GABA-LI in the terminals and throughout the mossy fiber pathway. These findings confirm that GABA-LI is present in the mossy fiber terminals under baseline conditions, and that merely incubating slices in standard Ringer solution, a common protocol in laboratories using hippocampal slices for electrophysiological and biochemical studies, is sufficient to greatly enhance GABA-LI in the mossy fiber pathway.

771.2

IMMUNOCYTOCHEMICAL LOCALIZATION OF 65- and 67kDa GLUTAMIC ACID DECARBOXYLASE (GAD)- AND GABA-LIKE IMMUNOREACTIVITIES (LI) IN THE RAT HIPPOCAMPAL FORMATION; GAD67- AND GABA-LI ARE PRESENT IN NORMAL DENTATE GRANULE CELLS AND ARE RAPIDLY INDUCED BY AFFERENT STIMULATION, KAINIC ACID, OR PILOCARPINE. R.S. Sloviter*, A.L. Sollas, E. Dean, and M.A. Dichter.
Neurology Research Ctr., Helen Hayes Hospital, W. Haverstraw, NY 10993, the Depts. of Pharmacology and Neurology, Columbia University, New York, NY 10032, and the Dept. of Neurology, University of Pennsylvania, Phila., PA 19104

Recent cell culture studies suggest that both excitatory and inhibitory cells contain mRNAs for GAD65 and GAD67. This implies that under certain conditions, excitatory neurons may produce GAD and GABA, and temporarily acquire inhibitory properties. We have tested this hypothesis by inducing seizure discharges **in vivo** and then localizing GAD65-, GAD67-, and GABA-LI. However, we unexpectedly discovered in pilot studies utilizing improved immunocytochemical methods for the detection of GABA-LI that dentate granule cell axon terminals of normal animals contain intense GAD67- and GABA-LI. Stimulation of the perforant path once per minute with 10sec-20Hz stimulus trains evoked population spikes and epileptiform discharges in the granule cell-, CA3-, and CA1 pyramidal layers. Six hours of afferent stimulation induced GAD67- and GABA-LI in granule cells, but not in hilar mossy cells or hippocampal pyramidal cells. Stimulation did not detectably alter GAD65-LI. Granule cell GAD67- and GABA-LI were also induced after kainate or pilocarpine-induced seizures. These results indicate that granule cells normally express GAD67 and GABA and that granule cell excitation increases their expression. These findings suggest that granule cells may normally possess both excitatory and inhibitory characteristics and that excessive excitation may temporarily shift the balance in favor of the inhibitory component, possibly altering hippocampal excitability, seizure frequency, severity, and duration as a result. Supported by NINDS grant NS18201.

771.3

EARLY GONADECTOMY MODULATES LONG-TERM POTENTIATION AT SCHAFFER COLLATERAL-CA1 SYNAPSES IN HIPPOCAMPUS. L. Velisek, J. Veliskova, A.M. Etgen, S. L. Moshé, P.K. Stanton*. Departments of Neurology and Neurosience, Albert Einstein College of Medicine, Bronx, NY 10461

Gonadal steroids play an important role in the development of the nervous system. In this study, we examined the gonadectomy-induced changes in Shaffer collateral-evoked synaptic transmission in CA1 hippocampal neurons in slices from 29-31 day old male rats castrated on day of birth. Sham operated littermates served as controls. In another study, we examined slices from adult females ovariectomized 7 days prior to the experiment, and given estrogen supplement two days prior the experiment and a single progesterone injection at day of the experiment. We observed no difference in the maximal population spike amplitude between naive slices from normal and castrated males. In slices from ovarectomized females given hormone supplement we found significantly larger maximal population spike amplitude than in controls (8.6±1.3 mV versus 4.8±0.7 mV). In both males and females, slices from gonadectomized rats exhibited significantly larger short-term potentiation 1-20 min following tetanization, compared to controls. In contrast, gonadectomy did not cause any difference in the amplitude of LTP measured 30 min after high-frequency stimulation (2x100Hz/0.5sec) in males or females.

The data suggest that gonadectomy causes a selective enhancement of short-term potentiation of synaptic transmission in hippocampal field CA1.

771.4

CONTINUOUS HIPPOCAMPAL STIMULATION-INDUCED STATUS EPILEPTICUS RESULTS IN ALTERATIONS IN MICROSOMAL CALCIUM UPTAKE. L. D. Kochan*[2], S.B. Churn[1], R.E. Blair[2], V. J. Obias[3], and R. J. DeLorenzo[1,2].
Department of Neurology[1], Department of Pharmacology and Toxicology[2] and Department of Physiology[3], Medical College of Virginia, Richmond, VA 23298.

Status Epilepticus (SE) is a seizure disorder associated with high mortality and morbidity and neuronal cell loss despite aggressive treatment. Hyperexcitability and neuronal cell death have been well correlated with prolonged increases in intracellular calcium. This has provoked interest in calcium homeostatic mechanisms such as the high affinity, low capacity microsomal Ca^{2+} ATPase. Alterations of this enzyme could contribute to the prolonged increases in intracellular calcium that ultimately accounts for the high morbidity and mortality associated with prolonged seizures. The Continuous Hippocampal Stimulation (CHS) model of SE was employed to study the effects of seizure activity on microsomal calcium uptake. Test animals received 'continuous' hippocampal stimulation consisting of 10-s trains of 50-Hz, 1-ms biphasic square wave pulses (400uA) every 11s for 90 minutes. Implanted/Sham animals were used as controls. Rat brain microsomes were isolated from control and SE animals by differential centrifugation for determination of microsomal calcium uptake. Microsomes prepared from control and SE animals displayed ATP-dependent Ca^{2+} uptake. However, CHS-induced SE resulted in significant inhibition of Ca^{2+} uptake, 47%, compared to control. The data indicate that SE causes alterations in microsomal calcium uptake and thus may play a role in causing elevated intracellular Ca^{2+}. The data support the hypothesis that alterations in microsomal calcium uptake may ultimately account for some of the morbidity and mortality associated with prolonged seizures.

771.5

FELBAMATE PREVENTS THE DEVELOPMENT OF KINDLING PRODUCED BY CHRONIC TREATMENT WITH PENTYLENETETRAZOL IN THE RAT. **M. Orlandi, O. Giorgi, F. Marrosu*[1], M. Spiga, V. Valentini and M.G. Corda. Departments of Toxicology and [1]Neurology, University of Cagliari, Italy.**

Chronic treatment with an initially subconvulsant dose of pentylenetetrazol (PTZ), a blocker of the Cl^- channel of $GABA_A$ receptors, determines the progressive development of seizures (i.e., chemical kindling). It has been proposed that an enhancement in NMDA receptor-mediated neurotransmission together with a decrease in GABAergic function play a role in PTZ-induced kindling (J. Pharmacol. Exp. Ther., 262, 792, 1992). Since the anticonvulsant effect of Felbamate (FELB) is probably mediated via interaction with the glycine site of NMDA receptors, it was considered of interest to examine the ability of FELB to antagonize PTZ kindling. Rats were treated with a subconvulsant dose of PTZ (30 mg/kg, i.p., every second day) for up to 8 weeks. Two other groups of rats received FELB (300 or 400 mg/kg, i.p., 90 min before each dose of PTZ) and control rats were treated chronically with saline (2 ml/kg., i.p.). Pretreatment with FELB prevented the development of PTZ kindling partially at the dose of 300 mg/kg and completely at 400 mg/kg (seizure score by the end of the chronic treatment, 0-5 scale: control, 0; PTZ alone, 3.23; PTZ + FELB (300), 1.13; PTZ + FELB (400), 0.21). FELB also prevented the increased sensitivity to the convulsant effect of GABA function inhibitors observed in PTZ-kindled rats. Thus, a challenge dose of isoniazid (120 mg/kg, s.c.) or PTZ (20 mg/kg, i.p.) 15 to 60 days after the end of the chronic treatment induced generalized convulsions in >80 % of PTZ-kindled rats and in <10 % of controls or rats treated with PTZ + FELB (400). The results support the view that NMDA receptor-mediated neurotransmission may be involved in the development of PTZ kindling.

771.6

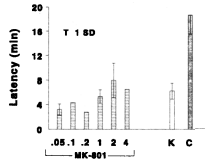
LIMBIC SEIZURES CAUSE CHRONIC SUSCEPTIBILITY TO STRESS IN RATS. Y. Wang[1], S. Ben-Eliyahu[1], G. Tocco[2], M. Mintz[1]* Psychobiology Research Unit, Department of Psychology, Tel Aviv Univ., Tel Aviv, Israel 69978. [2]Neuroscience program, Univ. of Southern California, CA.

Some patients with temporal lobe epilepsy (TLE) experience psychotic symptoms during the interictal periods of the disorder. The study of such interaction may further our understanding of psychotic behavior. Electrical kindling in animals, a process by which daily sub convulsive stimulation comes to elicit full clonic tonic limbic seizures, is used as a model for TLE. The long term effects of kindling include a decrease in dopamine (DA) reactivity in the prefrontal cortex and increased DA reactivity in the striatum. Such cortical-striatal pattern of DA imbalance may underlie some psychotic behaviors. Such pattern also predicts increased behavioral reactivity to stressful stimuli, a common symptom among psychotics. Therefore, in the present study, rats were electrically or sham stimulated once daily until 5 or 10 full clonic tonic seizures have occurred, and their susceptibility to stress was tested 4 weeks later. Kindled animals, as compared to sham-kindled and naive controls, showed increased locomotion in a novel environment and elevated analgesia on a hot plate test. Naloxone (10mg/kg) did not abolish the effect of kindling on the analgesic response. To further study the relationship between kindling and stress, binding to striatal DA-D1 (SCH-23390) and DA-D2 (YM-09151-2) receptors and to DA-uptake sites (GBR-12935) was assessed using autoradiography following acute restrainment stress. Acute stress caused increased DA-D2 receptor binding in the dorsal striatum in all groups. However, neither kindling nor the interaction of kindling and stress had significant effect on binding to DA receptors or DA uptake sites. In conclusion, the present result suggest that kindling increases susceptibility to stress, and the mechanism underlying this effect is not related to changes in DA receptors or DA uptake sites.

771.7

NMDA RECEPTOR ANTAGONISTS AGGRAVATE HYPERBARIC OXYGEN-INDUCED SEIZURES IN RATS. M. Chavko, A.L. Harabin, J.C. Braisted, S.L. Pocotte*. Naval Medical Research Institute, Bethesda, MD 20889-5607.

Excitatory amino acid receptor antagonists are anticonvulsant in a variety of seizure models. We studied the effect of two non-competitive NMDA receptor antagonists on the latency to electroencephalographic discharges in awake rats (male, Sprague-Dawley, 380-480 g) exposed to 5 atm O_2. MK-801 (0.05 to 4 mg/kg) (n= 12) or ketamine (K) (20 or 100 mg/kg) (n=6) injected ip 30 min before exposure markedly *shortened* latency to seizure when compared to saline-injected controls (C) (n=3) (Fig 1). Because MK-801and ketamine may increase cerebral blood flow (CBF), and increases in CBF may enhance O_2 seizures, we measured the effect of MK-801 on CBF. We chronically implanted a laser-Doppler flow probe over the dura of a separate group (n=4) of rats. MK-801 progressively increased CBF up to 300% between 0.05 and 1 mg/kg. The effect of MK-801 on susceptibility to O_2 seizures correlates with the increased CBF. The proconvulsant effect was slightly attenuated at higher doses (> 0.2 mg/kg) known to be effective in other seizure models. We propose two mechanisms by which MK-801 affects O_2 seizures: (1) A dominant proconvulsant effect mediated by increases in CBF and (2) A less powerful anticonvulsant effect operating at higher doses via a receptor-mediated mechanism.



(Supported by NMRDC Work Unit 61152N MR00001.001-1501)

771.8

NEGATIVE SHIFT IN DC-EEG LINKED TO A FALL IN END-TIDAL CO_2 DURING VOLUNTARY HYPERVENTILATION. J. Voipio*, K. Kaila, H. Tolvanen and E. Heinonen. Department of Biosciences, 00014 University of Helsinki, Finland.

We have examined the effects of hyperventilation-induced changes in CO_2 partial pressure (Pco_2) on cortical neuronal excitability in experiments on human volunteers (age 22-44 years, n=9). Bipolar recordings between the vertex (C_z) and the left mastoid were made using a purpose-designed DC-coupled EEG amplifier (bandwidth 0-160 Hz) and sintered Ag/AgCl electrodes. A 3-minute period of hyperventilation led to a mean fall in end-tidal Pco_2 from 38 to 24 mm Hg and to a negative shift of 578 ± 320 μV (± SEM), indicative of an increase in neuronal excitability. The inter-individual variation of the maximum DC shift ranged from 250 to 1250 μV while the individual variation in recordings repeated at intervals of 1-2 weeks was less than 10% (n=3). Voluntary apnea, which leads to a build-up of CO_2, produced a positive shift in the DC-EEG. When breathing a gas mixture of 5% CO_2 + 95% air, a respiratory pattern similar to that employed in air hyperventilation produced only small decreases in end-tidal Pco_2 (from 43 to 38 mm Hg) and a clear-cut inhibition (80-100%) of DC shifts when compared with the responses in air hyperventilation. The present data indicate a strict dependence of the negative shift in the DC-EEG on a decrease in Pco_2, which may be accounted for by the well-known enhancement of neuronal excitability induced by respiratory alkalosis that has been repeatedly documented in work on animal models and on in vitro preparations.

771.9

MODIFICATION OF KAINATE-INDUCED BEHAVIORAL AND ELECTROGRAPHIC SEIZURES FOLLOWING INHIBITION OF NITRIC OXIDE SYNTHASE IN MICE. R.D. Kirkby*, R.A. Forbes[1], and S. Subramaniam, Neuronal Excitability Section, Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892-1408 and [1]Department of Anesthesiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20810.

We assessed the effects of N^{ω}-nitro-L-arginine-methyl ester (L-NAME), an inhibitor of nitric oxide synthase, on kainate-induced seizures in adult male NIH Swiss mice. L-NAME dose-dependently shortened latency to convulsions following systemic kainate (44 mg/kg; s.c.) and increased the incidence of convulsive wild running (to the exclusion of the less severe clonic convulsions typically seen in controls); L-NAME did not shorten latency to kainate-induced afterdischarge (AD) in the hippocampus, amygdala, frontal cortex, or midbrain reticular formation. Also, 4 of 6 L-NAME-treated (5 mg/kg; i.p.) mice failed to express AD during initial fits of wild running, suggesting an uncoupling of electrographic and behavioral components of kainate-induced seizures. Finally, L-NAME shortened latency to wild running induced by intraventricular kainate (1 nmol) but altered neither behavioral (clonic) nor electrographic seizures elicited by intrahippocampal kainate (1 nmol). Because L-NAME potentiated seizures following either systemic or intraventricular administration of kainate but not intrahippocampal (focal) administration, the proconvulsant effects of L-NAME may depend on bilateral or extrahippocampal actions of kainate. Moreover, because L-NAME facilitates kainate-induced wild running, which bears striking similarities to motor components of audiogenic seizures in susceptible strains of rodents, it is possible that L-NAME influences tectopontine mechanisms that mediate audiogenic seizures.

771.10

•CAPTURE OF NITRIC OXIDE WITH HEMOGLOBIN IN BRAIN MICRODIALYSIS. • Y. Zhang, F.E. Samson, S.R. Nelson*, T.L. Pazdernik, Smith Research Center, Univ. of Kansas Med. Ctr., Kansas City, KS 66160.

• The role of nitric oxide (NO) in many physiological processes including neurotransmission, memory and brain injury is the focal point of many neurobiological studies. There is a need for a reliable method to directly monitor NO. Oxyhemoglobin (Hb) has high affinity for NO and upon binding is converted to MetHb quantitatively. Spectroscopy of Hb conversion to MetHb gives an effective measurement of NO concentration. Although this method is especially promising for microdialysis many factors can influence the reproducibility, sensitivity and stability of this assay, making it difficult to get reliable results at low NO levels. Factors such as protein stability, spectral data processing and temperature control, as well as microdialysis parameters were investigated. Also, direct monitoring of NO in microdialysates from rat brain was assessed. By measuring the difference in the diffusion rates of NO and sodium nitroprusside across the microdialysis membrane, we found that NO readily diffuses through the membrane. An optimal approach to measuring the conversion of Hb to MetHb is to use an integral of the difference spectrum which accounts for both the net increase in MetHb and net decrease in Hb. Another critical step is to take the isobestic point as the reference point to "zero" the spectra. This not only eliminates a serious error source from protein degradation but also compensates for unpredictable reflection effects due to small volumes (50-60 µL). This assay has a practical detection limit of about 10 nM (0.5 pmol/sample) in the microdialysate. We evaluated the method in anesthetized rats by demonstrating that the excitotoxin kainic acid (13 mg/kg, ip) induced NO release (17±7 nM). This study developed a protocol with detailed analytical parameters for No monitoring in neurobiomedical research. Supported by DAMD17-94C-4045.

771.11

INCREASED PURINE LEVELS IN THE HIPPOCAMPUS MEASURED BY MICRODIALYSIS FOLLOWING SEIZURES. R. F. Berman* and Bertil B. Fredholm. Wayne State University, Detroit, MI and Karolinska Institutet, Stockholm, Sweden.

There is pharmacological evidence that adenosine is an endogenous anticonvulsant agent. We therefore measured brain levels of purines (e.g., adenosine, inosine, guanosine, xanthine, and hypoxanthine) by microdialysis before and after seizures. Seizures were produced in anesthetized male rats by injection of either bicuculline (0.5 mg/kg, i.v.) or kainic acid (12.0 mg/kg, i.v.), and were monitored electrophysiologically via an electrode implanted, unilaterally, into the hippocampus. A 1.0 mm microdialysis probe was implanted in the other hippocampus. Purine levels were quantified by HPLC. Levels of purines increased substantially during seizures, with the largest increases seen for hypoxanthine and inosine. Smaller increases were seen for adenosine. These effects were observed with both kainate and bicuculline seizures. Addition of the adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) to the dialysis media resulted in higher basal levels of adenosine, and larger increases in adenosine during seizures. Basal levels of the adenosine metabolite inosine were correspondingly lower after the addition of EHNA, and increases during seizures were of lower magnitude. These findings indicate that brain levels of adenosine increase during seizures, with concomitant increases in levels of adenosine metabolites. The results support the hypothesis that increased adenosine levels may contribute to modulation of seizure activity, and may play a role in the termination of ongoing seizures and the patterning of subsequent seizures. (Supported by the Swedish Medical Research Council).

771.12

PATTERN OF DISTANT NEURONAL DAMAGE INDUCED BY AMPA-RECEPTOR MEDIATED STATUS EPILEPTICUS EVOKED FROM AREA TEMPESTAS. A. Easton*, D.Belcher, D. Masco, K. Gale, N. Sahibzada. Georgetown University Medical Center and University of DC, Washington D. C.

Cyclothiazide (1.8 nmol), an agent preventing desensitization of AMPA receptors, when focally applied concurrently with bicuculline methiodide (118 pmol) into the area tempestas (AT) results in a prolonged (1-7 hr) status epilepticus (SE) which can be terminated by application of an AMPA receptor antagonist, NBQX (500 pmol) in AT. To evaluate the pattern of neuronal injury in this model, rats were sacrificed 2-3 days after SE and the brains processed using silver impregnation. With SE lasting 2-3 hr, pronounced neuronal degeneration occurred bilaterally in the anterior olfactory nucleus, endopiriform nucleus, amygdaloid nuclei, insular, piriform and rhinal cortices, hippocampus and several thalamic nuclei. The pattern of degeneration was similar in many respects to that produced by systemic kainic acid. In rats with brief periods of SE (1 hr), neuronal damage was largely restricted to the hemisphere from which the seizures were initiated. Under these conditions, there was a notable absence of degeneration in the hippocampus. Otherwise, the brain regions showing unilateral degeneration closely matched those affected bilaterally in rats with more prolonged SE. It appeared that involvement of the hippocampus was closely associated with, and possibly required for, degeneration of regions in the contralateral hemisphere.

These results show that SE evoked focally from AT leads to a pattern of brain damage resembling that seen with limbic SE evoked by systemic chemoconvulsants. This model of focally-evoked SE is highly controllable, with a clearly defined onset, and has the advantage of having the drug treatment confined to a small area, remote from most of the damaged cell populations. Supported by NIH grants NS28130 and GM08005.

771.13

BOTH EXCITOTOXIC AND APOPTOTIC MECHANISMS MAY UNDERLIE BRAIN DAMAGE INDUCED BY INJECTION OF AMINOOXYACETIC ACID INTO THE RAT ENTORHINAL CORTEX. [1]F. Du* and [2]T. Eid, [1]Maryland Psychiatric Research Center, Baltimore, MD 21228 and [2]Department of Anatomy and Cell Biology, University of Bergen, Bergen, Norway.

Injection of aminooxyacetic acid (AOAA), an indirect excitotoxin, into the rat entorhinal cortex (EC) causes a seizure-related neuronal loss in EC layer III (Neurosci Lett. 147:185, 1992). In order to determine whether this distinct neurodegeneration involves apoptosis, we examined AOAA-injected rats using Nissl staining and an *in situ* DNA nick end-labeling technique (TUNEL; J. Cell Biol. 119: 493, 1992) at 24 or 48 hours, or 5 days after the AOAA injection. AOAA treatment resulted in intense nuclear TUNEL labeling in neurons within several limbic regions ipsilateral to the injection. At 24 hours, labeled neuronal nuclei were mainly detected in the most medial region of layer III in the ventro-medial EC and in the lateral amygdaloid nucleus. At 48 hours, many labeled nuclei were observed not only in layer III of the entire medial EC and in the lateral amygdaloid nucleus, but also in the subiculum. This distribution pattern of TUNEL-labeled nuclei remained similar after 5 days. Moreover, morphological characteristics of apoptosis, such as condensation of chromatin and nuclear fragmentation, were noticed in the regions described above. Our results indicate that, in addition to the excitotoxic mechanism previously suggested, a delayed apoptotic cell death is involved in AOAA-induced neurotoxicity in limbic regions. The functional consequences of the dual mechanisms underlying these AOAA-induced lesions are currently under investigation.

Supported by a grant from the Epilepsy Foundation of America.

771.14

INHIBITION OF MICROGLIA ACTIVATION REDUCES CORTICAL NEURONAL CELL LOSS AFTER STATUS EPILEPTICUS. L.S. Chen* and O.C. Snead III. Div. of Neurology, Childrens Hospital Los Angeles, University of Southern California. Los Angeles, CA 90027

Status epilepticus (SE) is associated with acute neuronal injury, which can activate microglia. The activated microglia are capable of killing neurons by releasing neurotoxic factors. Therefore, seizure-induced neuronal cell loss (NCL) may have two components: seizure-induced primary NCL and microglia-induced secondary NCL. To test this hypothesis, we studied the effect of inhibiting microglia activation on NCL after SE. Rats were given intrastriatal injection of 0.1 μmole kainic acid to induce SE or vehicle for control (n=6). Neuronal cell density (NCD) was calculated in deep cortical layers. %NCL was defined as: the difference of mean NCD between experimental and control groups/mean NCD of controls. Microglia were identified by C3 receptor (OX-42) immunohistochemically. SE produced progressive cortical NCL. The %NCL was 49% (n=6) at day 1, 67% (n=6) at day 3 and 90% (n=6) at day 7, despite the cessation of seizures at day 1 or day 2. The progressive NCL was correlated with a progressive increase of microglia in the cortex. Administration of colchicine (0.2 mg/kg/day, i.v.) and chloroquine (2.5 mg/kg/day, i.m.) decreased OX-42 immunoreactivity and reduced NCL ($p < 0.01$). The % NCL was 36% at 7 days after SE in rats (N=5) treated from day 1-4; and was 51% in rats (n=6) treated from day 4-7. These data suggest that microglia activated after SE significantly contribute to NCL. Early intervention to inhibit microglia activation can reduce NCL after SE.

771.15

REGENERATION AND HYPEREXCITABILITY IN HIPPOCAMPAL CULTURES AFTER TRANSECTION OF THE SCHAFFER COLLATERAL PATHWAY.

R.A McKinney, B.H. Gähwiler* and S.M. Thompson

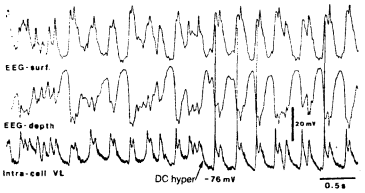
Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland

Seizures are a common consequence of head trauma, and it has been suggested that axonal reorganization may underlie lasting changes in excitability. Mature organotypic hippocampal cultures (>14 days *in vitro*) were used to investigate the capacity of recurrent axon collaterals of CA3 pyramidal cells to sprout in response to transection of the Schaffer collateral pathway with a razor blade. Cultures were permitted to survive for varying times before either injecting biocytin, to observe axonal arborization, or performing GAP-43 immunohistochemistry to visualize regenerating axons. Quantitative changes in dendritic spines were investigated by injecting CA1 cells with Lucifer Yellow and imaging by confocal microscopy. Axonal sprouting within area CA3 was evident at 3 days post-lesion. By 14 days many axons possessed growth cones and were found to have grown across the cut to the CA1 region. At 3 days post-transection, dendritic spines on CA1 cells were elongated and their number began to diminish 7 days post-lesion. Interestingly, spine number returned to control values by 21 days. Functional synaptic excitation of CA1 cells by stimulation in area CA3 was recorded at <14 days post-lesion. Sprouting axons exhibited more swellings, i.e. putative presynaptic boutons, compared to control axons. Evidence of an increase in the strength of excitation relative to inhibition within area CA3 was apparent, thereby suggesting a possible role for axonal sprouting in post-traumatic epilepsy.

771.16

THALAMOCORTICAL RELATIONS DURING TRANSITION FROM SLEEP TO EPILEPTIC PATTERNS IN CATS. N. Dürmüller*, D. Contreras and M. Steriade. Lab. Neurophysiol., Sch. Med., Laval University, Quebec, Canada G1K 7P4.

When spike-wave (SW) paroxysmal activity spontaneously develops from sleep patterns in corticothalamic circuits of anesthetized cats, a surprisingly high number of thalamocortical (TC) neurons are hyperpolarized and remain silent throughout the seizure (Steriade and Contreras, 1995). To shed further light on the behavior of such TC cells, we performed intracellular recordings of thalamic and cortical neurons and simultaneously recorded the surface- and depth-EEG activity from the related cortical areas. During SW seizures, the membrane potential of TC cells repolarized between rhythmic EPSPs, in close temporal relation to the depth-positive EEG wave. This repolarization decreased with depolarizing DC, and was enhanced by hyperpolarizing DC, thus suggesting a non-GABAergic origin. The repolarization was not enough to de-inactivate the Ca^{2+} current underlying spike-bursts and most TC cells remained silent. With slight hyperpolarizing DC injection, cells fired typical spike-bursts at each depth-negative EEG wave (see figure). With depolarizing DC, TC neurons fired short spike-trains that could be easily confounded with spike-bursts in extracellular recordings. We propose that the absence of burst firing in a large proportion of TC cells is due to the lack of a GABAergic component characteristic for other forms of SW seizures. Supported by MRC of Canada (grant MT-3689) and the Savoy Foundation.



EPILEPSY: BASIC MECHANISMS VI

772.1

A 4 PROCESSOR PARALLEL COMPUTER USING DIGITAL SIGNAL PROCESSOR CHIPS (DSP). APPLICATIONS IN REAL TIME TOPOGRAPHIC MAPPING OF CORTICAL AREAS DURING AMYGDALOID KINDLING IN THE CAT. R. Fernández Mas and A. Fernández-Guardiola*. Div. Invest. en Neurociencias, Inst. Mexicano de Psiquiatría. México 14370, D.F. México.

We describe the application of a custom designed parallel processor in real time digital signal analysis in experimental epilepsy. Our main goal is to analyze the temporal characteristics of the propagation of afterdischarges induced in the temporal lobe amygdala to the cerebral cortex. This processor is able to generate a "cartoon" of the propagation dynamics in order to characterize the afterdicharge with a very high temporal resolution, and study the evolution of each spike in the cortex. As the Kindling develops, the afterdischarge becomes wider and complex in terms of the dynamics of propagation.

The method generates a series of color maps which shows the temporal evolution of selecte spikes. To satisfy the computational requirements, we design a parallel processor based on 4 DSP (digital signal processor) chips with a total 80 million instruction per second. Thus can be generated one map in one millisecond, allowing the real time tracking of ictal and interictal phenomena. Each processor also has 4 analog input channels. This scheme yields an efficient process distribution among processors resulting in a very fast achievement of the interpolation and map generation algórithms. Partially Supp. by DGAPA-UNAM.

772.2

PACING A NEURONAL NETWORK WITH PULSED ELECTRIC FIELDS. T. Netoff, D.H. Duong and S.J. Schiff *. Department of Neurosurgery, Children's National Medical Center and George Washington Univ., Washington, D.C. 20010.

Endogenous electric fields of active neurons are known to participate in neuronal synchronization. In 8.5 mM [KCl], *in vitro* hippocampal brain slices exhibit neuronal population burst-firing that shares physiological similarities with interictal epileptic spikes. We have recently demonstrated that such population activity could be paced with orthodromic and antidromic stimulation of the tissue (*J. Neurophys.* 73: 876-879, 1995).. We sought to determine whether such entrainment could be replicated noninvasively with electric fields.

Transverse slices 400 μm thick were prepared from the hippocampus of 125-150 gm female Sprague-Dawley rats with a tissue chopper, and placed in an interface type perfusion chamber at 32-36.5 ^{o}C. Autonomous burst firing was induced with 8.5 mM [KCl] in the perfusate. Pulsed dipole electric fields were created using 2 platinum plate electrodes placed 1 cm apart within the perfusion bath with a tissue slice in between. Extracellular recordings were performed with micropipette electrodes.

Twenty-two experimental trials were performed on 22 slices from 21 rats. Square wave electric field pulses were delivered in trains, 0.75-1.3 Hz, 0.3-1.0 msec duration, delivering brief steady state fields of 15-83 mV/mm. In each experiment, by increasing the field strength, pulse duration, or both, the response of the neurons could be entrained into a 1:1 relationship with the field pulses. At higher intensities of pulse strength and duration, strict time locking of stimulus and response was seen. At pulse frequencies above the natural interburst interval of the tissue, more complex responses could be observed.

These results demonstrate that a burst-firing autonomous neuronal network can be entrained with relatively small pulsed electric fields. These results may be useful in the control of such systems

772.3

NONLINEAR CHAOTIC AND NON-AUTONOMOUS PERIODS IN HIPPOCAMPAL SEIZURES. J. Csicsvári*, A. Bragin and G. Buzsáki. CMBN, Rutgers University, Newark, NJ 07102

A 16-channel silicon probe array was used to record field potentials during seizures in the hippocampus. Current source density (CSD) analysis was applied to eliminate the effect of volume conduction. In the CSD profiles the wave components (< 50 Hz) were analyzed. An amplitude vs. depth vector was constructed by collecting the peak amplitudes from each CSD channel. Sammon's non-linear projection technique was used to visualize the spatial distribution of the amplitude vectors in their higher dimensional feature space. The multidimensional location of each amplitude vector was projected and plotted in three dimension. This method enabled us to examine the quasi-trajectory of the epileptic afterdischarges. Two different states were observed in these plots: attractor state and a continuously changing state (CCS). In the attractor state the trajectory randomly changed within a subvolume of the feature space while in the CCS the consecutive amplitude vectors usually formed a curve changing toward a definite direction and showing certain linear tendencies. The activation patterns of these states were similar in different animals. Typically, the initial CCS phase was followed by an attractor phase. This first attractor state, representing the main part of the afterdischarge, turned into a complex CCS. This complex state usually contained more than one CCS segment. Seizures were terminated in an elongated attractor phase. Traditional non-linear dynamical analysis methods were also performed (fractal dimension calculations on original and randomized signals and recurrence plots) and they indicated chaotic dynamics during the attractor phase and non-autonomous dynamics during the CCS. These methods are useful for the classification of spatially-temporally distinct patterns.

772.4

HIPPOCAMPAL AFTERDISCHARGES IN THE INTACT RAT: ROLE OF COUPLED OSCILLATORS. A. Bragin*, J. Csicsvári, M. Penttonen and G. Buzsáki. CMBN, Rutgers University, Newark, NJ 07102

Epileptic field patterns and unit activity evoked by perforant path (PP) or commissural (COM) stimulation were monitored in the CA1-dentate axis, CA3 and entorhinal regions using multisite silicon probes in freely moving rats. Both stimulation sites induced 2-6 Hz rhythmic afterdischarges (AD) with superimposed gamma (40-100 Hz) oscillations and 200-400 Hz population discharges. PP-induced seizures were initiated in the entorhinal cortex-dentate gyrus axis and later entrained the CA3-CA1 regions, whereas COM stimulation triggered afterdischarges in CA3-CA1 and entrained the entorhinal-cortex-dentate gyrus. During the main part of the seizure all regions discharged synchronously often without any apparent lead by any of the regions. Following bilateral removal of the entorhinal cortex the basic pattern of AD in the CA3-CA1 axis remained similar. The primary afterdischarge always terminated first in the CA1 region, heralded by a slowly spreading (0.1 mm/sec), low amplitude, 70 Hz oscillation. Population burst initiation migrated within the CA3c-a axis. Non-synaptic population discharges of CA1 pyramidal cells were occasionally observed. Basket cells discharged primarily during the population spikes whereas other interneurons sustained long trains of fast (>200 Hz) spike trains. We suggest that two seizure inducing systems exist in the intact brain: the CA3-CA1 circuitry and the entorhinal-dentate network. These oscillating circuitries are normally coupled, but they may also sustain afterdischarges independently.

772.5

DYE COUPLING OF NEURONS IN THE HIPPOCAMPUS IMPLIES A ROLE FOR GAP JUNCTIONS IN EPILEPSY. M Penttonen*, A Bragin, A Sik, and G Buzsaki. CMBN, Rutgers University, Newark, NJ 07102

In awake rats hippocampal epileptic seizures first terminate in the CA1 region by a slowly spreading (0.1 mm/sec) (60-80 Hz) fast oscillation. A likely mechanism responsible for the oscillation is electrical coupling of pyramidal neurons by gap junctions (Bragin et al.; this meeting). In this experiment we tested this hypotheses by intracellular injection of biocytin or neurobiotin into pyramidal neurons in urethane anesthetized rats. Epileptic afterdischarge was induced by 200 Hz stimulation of commissural pathways or by local micropressure ejection of KCl after dye injection and withdrawal of the pipette from the cell. Commissural stimulation or KCl ejection induced a large intracellular depolarization coupled with extracellular -DC shift. Rhythmic afterdischarges erupted during recovery from depolarization. In intact rats <3% of the injected neurons showed coupling. In rats with afterdischarges >40% of the labeled pyramidal cells were dye coupled with other pyramidal cells, interneurons or astrocytes that were found close to the filled pyramidal neurons. Electronmicroscopic investigation of gap junctions between dye coupled cells is under way. We hypothesize that gap junction-mediated cell coupling and associated decrease of the input resistance may be responsible for the termination of epileptic activity.

772.6

LAMINAR AND TOPOGRAPHICAL ANALYSIS OF PERIRHINAL CORTEX KINDLING IN THE RAT. L.L. Felstead*, M.E. Kelly and D.C. McIntyre. Psychology Dept., Carleton Univ., Ottawa, Ont., Canada, K1S 5B6.

Recently we reported that the rate of kindling in the anterior perirhinal cortex was faster than all other limbic structures. Equally important, the latencies to forelimb clonus from stimulus onset of these perirhinal convulsions were extremely brief (<1 s). These two results suggested that the perirhinal cortex might have direct access to motor systems. Our subsequent anatomical studies confirmed this prediction by showing dense perirhinal projections to the frontal motor cortex, originating principally in the layer V cells. Also, we observed that the entire rostrocaudal extent of the perirhinal cortex projected to the frontal cortex. In the present study, we determined whether the laminar differences we observed in the perirhinofrontal projections were associated with differences in kindling profiles when the kindling electrodes were restricted to different perirhinal laminae. In addition, we assessed whether the entire rostrocaudal extent of the perirhinal cortex shared the previously observed rapid kindling/short latency profile. The results indicated that kindling electrodes located in the perirhinal layers V/VI produced kindling rates of 1-2 trials compared to rates of 4-8 trials when electrodes were located superficially in layers I/III. Further, the rapid genesis of kindling was characteristic of the entire rostrocaudal extent of the perirhinal cortex. This laminar and topographical analysis of the perirhinal cortex provides further support for the view that this cortex might serve as the main conduit for limbic discharges to gain access to the motor structures that support limbic kindled convulsions.

772.7

INTRINSIC RESPONSES AND MORPHOLOGICAL FEATURES OF NEURONS IN THE RAT PERIRHINAL CORTEX. R.A. Battye* and D.C. McIntyre. Psychology Dept., Carleton Univ., Ottawa, Ont., Canada K1S 5B6.

Recent interest in the perirhinal cortex results from its involvement in memory, and its ability to rapidly develop kindled seizures with short latencies. The seizure data suggested direct connections between the perirhinal cortex and motor systems. We confirmed this suggestion in anatomical studies, where layer V cells were shown to project densely to the frontal motor cortex. Although little was known about these cells, Beggs & Kairiss (*Brain Res.*, 665:18-32, 1994) recently described several features of perirhinal cells that we confirm here and extend. In the present study, intracellular recordings were made from over 60 perirhinal cells in a coronal slice preparation (McIntyre & Wong, *J. Neurophysiol.*, 55:1295-1307, 1986). Intrinsic membrane responses were studied with depolarizing and hyperpolaring current steps. Subsequently, most cells were injected with 1-2% biocytin to visualize their morphology. 'Regular spiking' was the most characteristic feature of the small, layer III pyramidal cells. Additionally, these dendritically-slight layer III cells showed no anodal break response after hyperpolarizing current injection. Conversely, the large, layer V pyramidal cells frequently displayed 'intrinsic bursting' to depolarizing current injection at their resting potential, which changed into single spike mode when their membrane was held at more depolarized values. These dendritically-extensive layer V cells always showed a depolarizing anodal break response. It is these intrinsic bursting layer V cells that project extensively to the frontal motor cortex and may be responsible for projecting limbic discharges into their convulsive form.

772.8

LATERALIZATION OF AMYGDALA KINDLED CONVULSIONS BY UNILATERAL CORTICAL SPREADING DEPRESSION. M.E. Kelly *, R.A. Battye and D.C. McIntyre. Psychology Dept., Carleton Univ., Ottawa, Ontario, Canada K1S 5B6.

Kindling in the rat forebrain results in development of motor seizures that involve bilateral forelimb clonus. Several investigators have postulated that the motor substrates that support and drive these clonic responses may reside in the frontal cortex. It is known that all limbic sites have direct or indirect access to the frontal motor regions, and that transection of the anterior half of the corpus callosum is successful in lateralizing kindled motor seizures to the limbs contralateral to the kindled site. In the present study, we determined the involvement of the frontal cortex in the expression of forelimb clonus triggered during amygdala kindling in the rat. One day following the third stage-5 convulsion, spreading depression (SD) was induced in the frontal cortex either ipsilateral or contralateral to the kindled amygdala. SD was achieved via a 2 μl injection of 25% KCl into an intracranial cannula permanently positioned above the dura mater over the frontal cortex. Three min following the infusion of KCL, the amygdala was stimulated at its previous afterdischarge (AD) threshold. It was observed that SD in the frontal cortex contralateral to the stimulated amygdala lateralized the clonic convulsion, similar to split-brain rats, without affecting the AD duration. Conversely, SD in the ipsilateral frontal cortex blocked the convulsion and truncated the amygdala AD. These data strongly implicate the frontal motor regions as (a) critical to the manifestation of the forelimb clonus during limbic kindled seizures and (b) important in the elaboration of the ipsilaterally-triggered amygdala AD.

772.9

RADIAL ARM MAZE PERFORMANCE AFTER PARTIAL HIPPOCAMPAL KINDLING L. Stan Leung*, D. Brzozowki, D. Young and B. Shen Dept. Physiology and Clinical Neurological Sciences, University of Western Ontario, London, N6A 5A5, Canada.

In previous studies (Behav Brain Res 40: 11, 1990; Hippocampus 4: 696, 1994), we found that rats that were previously trained on a radial arm maze (RAM) with external cues showed a 'retention' deficit for up to 25 days after partial kindling of the hippocampal CA1 (15 afterdischarges (ADs) over 3 days). The partially baited 8-arm RAM was used in the following 3 experiments: (1) to find the minimal number of ADs required for RAM deficits; (2) to test if RAM deficits are also found in an internal cue maze; and (3) to test whether acquisition is affected by kindling. For the first two experiments, rats were trained on an external cue RAM with 4 arms baited, and rested for a week before partial kindling. In the 1st experiment, RAM deficits, mainly in reference memory, were found in rats within 1 week after 5 or 10 hippocampal ADs, and at 3 weeks only after 10 ADs. In the 2nd experiment, the rats were trained on an external cue maze and an internal cue maze before kindling. The internal cue maze had minimal extramaze cues, and the 4 baited arms with internal cues (e.g. carpet, sandpaper, etc. on the floor) were shuffled before each trial. After partial kindling (15 ADs), rats showed deficits only on the external but not the internal cue maze, consistent with the literature on hippocampal lesion. In the 3rd experiment, rats were first trained on a RAM in one room, then kindled, and acquisition on the RAM with a different set of baited arms was conducted in a new room. There was no difference in the acquisition rates between kindled and control rats. In conclusion, partial hippocampal kindling disrupted the performance of a previously learned external cue RAM but not that of an internal cue RAM. The acquisition of a new RAM does not seem to depend on whether rats are partially kindled or not. (Supported by NS 25383).

772.10

MOSSY FIBER SPROUTING AND LOW-FREQUENCY (RAPID) KINDLING. L.L. Armitage*, E.M. Jenkins, & M.E. Corcoran. Dept. of Psychology, University of Victoria, POB 3050, Victoria, BC, Canada V8W 3P5.

Anomalous sprouting of mossy fibers has been detected in the supragranular area of the dentate gyrus after kindling or kainic acid-induced seizures and in tissue specimens taken from patients with epilepsy. Recent findings suggest that mossy fiber sprouting is detectable during the early stages of kindling and becomes more prominent with the development of convulsive seizures (Cavazos et al., 1991). Because sprouting and conventional kindling have similar time courses, it is difficult to determine whether sprouting is invariably correlated with kindling. To address this issue, we used a rapid (low-frequency) kindling paradigm to determine whether the time courses of sprouting and kindling could be dissociated.

We implanted bilateral electrodes into the amygdala of rats. Low-frequency (3 Hz) electrical stimulation was administered once daily until the first generalized stage 5 seizure was kindled. Twenty-four hours later, brains were prepared for Timm sulfide-silver staining. Yoked control rats carried electrodes and were handled but did not receive stimulation. Although sparse Timm granules were observed in the supragranular area, the amount of mossy fiber sprouting did not differ between kindled and control rats.

Our results suggest that rapid kindling of one generalized stage 5 seizure can occur in the absence of prominent mossy fiber sprouting.
(Supported by the Medical Research Council of Canada)

772.11

MOSSY FIBER SPROUTING AND PERFORANT PATH KINDLING IN THE RAT FOLLOWING TRANSECTION OF THE FIMBRIA-FORNIX. P. Mohapel*, D.K. Hannesson, L.L. Armitage & M.E. Corcoran. Dept. of Psychology, University of Victoria, POB 3050, Victoria, BC, Canada V8W 3P5.

The anomalous sprouting of mossy fibers from dentate granule cells has been identified in human epileptics and several seizure models. It is unknown whether mossy fiber sprouting occurs following subcortical denervation of the hippocampus (HPC), via transection of the fimbria-fornix (FF). Previous research has demonstrated permanent changes in epileptic electrical activity, neurochemistry, and morphology in the HPC following FF lesions (Buzsáki et al.,1989; Lahtinen et al.,1993). In our first experiment, we attempted to map out the distribution of Timm granules in the supragranular region of the HPC and to plot the time-course of sprouting development following FF transection in rats. Mossy fiber sprouting was first evident, only in the dorsal aspect of the HPC, at about 14 days post FF lesion. The distribution of Timm granules was of moderate proportions with a continuous yet patchy pattern of granules being observed in the supragranular region between tips and crests of the dentate gyrus. The degree of sprouting did not appear to be greater at 30 days post lesion.

In a second experiment we implanted bilateral electrodes into the perforant path, the primary cortical pathway into the HPC, 30 days following FF lesions. Kindling stimulation was administered daily until generalized seizures were elicited. Afterdischarge thresholds were significantly lower in FF lesioned rats, but we found no significant differences in kindling rates between FF lesioned and non-lesioned rats. FF lesioned kindled rats exhibited a continuous and dense band of Timm granules that spanned into the inner molecular layer and was consistent throughout the entire HPC. Non-lesioned kindled rats demonstrated only moderate sprouting, similar to the degree observed with non-kindled lesioned rats. These results suggest that FF transections and perforant path kindling can independently induce mossy fiber sprouting, and in combination produce additive effects. Supported by MRC.

772.12

INHIBITION OF AMYGDALA KINDLING IN THE RAT BY CHRONIC METABOTROPIC GLUTAMATE RECEPTOR (mGluR) BLOCKADE. R.L. Beach*, R.B. Meeker, B.A. Thiede, X.Y. Xiong, and R.S. Greenwood. Department of Neurology, University of North Carolina, Chapel Hill, NC 27599

Although N- methyl-D-aspartate (NMDA) receptor activation appears to play a role in the development of the kindled state, the second messengers involved and the role of mGluRs in the process have not been elucidated. We kindled rats using daily 200 μA biphasic pulse trains, administered via stereotactically implanted amygdala electrodes. The selective mGluR antagonist (+)-alpha methyl-4-carboxyphenyl glycine (+MCPG) was chronically infused intracerebroventricularly via osmotic minipumps. Compared with the stereoisomer and drug free controls, +MCPG rats had a significantly decreased rate of kindling. +MCPG rats required significantly more stimuli to reach stage 5. +MCPG rats also required more stimuli to reach stage 3 seizures. At the time when all but one control animal were fully kindled, 50% of the mGluR animals had not reached stage 5 seizures. Afterdischarge durations (ADs) were shorter in the +MCPG rats at all time points, but were similar when compared by kindling stage. Interestingly, initial afterdischarge thresholds were moderately decreased by mGluR blockade. There was no evidence for adverse effects on the animals, as measured by behavior, water intake and body weights. These studies suggest an important role for mGluRs in mediating the plastic changes required for the development of the kindled state. Recent data suggests that NMDA and mGluR receptors cooperate in mediating neuronal plastic changes. The mechanisms via which NMDA and mGluR activation enhance seizure propogation and kindling, as well as the potential for synergistic effects are under further study.

772.13

QUENCHING: INHIBITION OF DEVELOPMENT AND EXPRESSION OF AMYGDALA KINDLED SEIZURES WITH LOW FREQUENCY STIMULATION S.R.B. Weiss*, X.L. Li, J. B. Rosen, H. Li, T. Heynen, R. M. Post Biological Psychiatry Branch, NIMH, Bethesda, Md. 20892

Using low frequency stimulation parameters, similar to those that induce long-term depression (LTD) or depotentiation *in vitro*, we attempted to alter amygdala kindling *in vivo*. Male Sprague Dawley rats were implanted with electrodes in the left amygdala, and kindled once/day with 60Hz, biphasic square wave pulses for 1 sec at their afterdischarge (AD) threshold. Immediately after the cessation of afterdischarge or seizure activity, "quenching" stimulation was administered through the same electrode for 15 minutes at a frequency of 1 Hz, using a 0.1 msec pulsewidth, and an intensity of 100μA over the AD threshold. Controls received either no current (sham-stimulation) or high frequency stimulation (100 Hz, .01 msec pusewidth, 100μA + AD threshold) for 15 minutes. All of the sham- (n=4) and high-frequency-stimulated (n=4) animals kindled, developing increasing afterdischarges and seizures within 10-14 days of stimulation. In contrast, 7 of the 8 rats that received quenching stimulation did not exhibit afterdischarge or seizure progression, although all of these rats exhibited ADs on day 1 of stimulation. The afterdischarge thresholds of the kindled control groups decreased or stayed the same after kindling, while they markedly increased in the animals that were quenched. This effect persisted for several weeks after quenching was discontinued and kindling stimulation resumed. In fully kindled animals, a week of quenching (without concurrent kindling) resulted in an increase in the AD threshold and a long lasting (>1 week and up to 7 weeks in some animals) decrease in the seizure response to the original kindling stimulus. This did not occur in animals given sham stimulation for one week.

These findings demonstrate that stimulation using LTD-like parameters *in vivo* has profound effects on amygdala kindled seizure development, expression and thresholds. Whether quenching is mechanistically related to either LTD or depotentiation requires further study. The marked and long-lasting effects of quenching suggest possible clinical applications not only for epilepsy, but also for other neuropsychiatric disorders where threshold phenomena may be crucial to illness emergence.

772.14

ESSENTIAL AMINO ACID (AA) DEFICIENCY LOWERS SEIZURE THRESHOLD IN THE RAT: A NOVEL FORM OF KINDLING? D.W. Gietzen*, K.D. Dixon, B.G. Truong, J.A. Barrett, A.C. Jones and D. Washburn. Dept Vet Anat Physiol & Cell Biol and Food Intake Lab. UC Davis, Davis CA 95616.

Neurotransmitter AAs, including GABA, glu, asp and gly, and disruptions of AA metabolism have been implicated in the neurochemistry of seizures, but nutritionally essential AAs have attracted less attention. The anterior piriform cortex (APC), aka "Area Tempestas" is both highly excitable (Piredda & Gale Nature 317: 623 '85) and activated by acute essential AA deficiency (Gietzen J Nutr 123: 610 '93). Repeated activation of the APC in chronic dietary AA deficiency could cause kindling. Therefore, we tested seizure threshold to pentylenetetrazol (PTZ) given ip, and to bicuculline (BIC) injected into the APC, in essential AA deficient (DEF) rats. In 7 trials, rats were fed defined diets with L-AAs (devoid of a single essential AA) as the sole protein source for at least 2 weeks before testing. The limiting AAs (number of trials) were: his (3), ile (2) and thr (2). Subthreshold doses, PTZ: 25 mg/kg + 5 mg/kg increments or BIC: 56 pmol + 10 pmol increments were given until seizures, stage $\geq$ 2, were observed for at least 5 sec. In the last 2 trials, pair-fed groups were included to control for an energy deficit due to reduced intake of the diets. Over all 7 trials, the AA DEF rats had reduced seizure threshold, as seen in lower doses of PTZ or BIC required to induce seizures, more severe seizures at subthreshold doses, and decreased latency to seizure. In trial 6, Thr DEF rats (N=8/gp) tested with PTZ ip had significantly shorter latency to stage $\geq$ 2 seizure (88$\pm$8 min vs 111$\pm$7 min for the control and 118$\pm$9 min for the pair fed group, $p = 0.05$). In trial 7, ile DEF rats given BIC into the APC reached $\geq$ stage 2 seizures at 72.9$\pm$3.6 pmol, while control and pair fed groups required $\geq$ 90.0$\pm$7.6 pmol to reach the same seizure level ($p \leq 0.05$). Thus, his, ile and thr deficiencies each increased seizure susceptibility in the rat. Supported by the Epilepsy Foundation of America; NIH DK35747 to the Food Intake Lab via UC Davis CNRU.

772.15

RGDS TETRAPEPTIDE FACILITATES HIPPOCAMPAL *IN VITRO* KINDLING: EVIDENCE FOR INTEGRIN MEDIATED PHYSIOLOGICAL STABILITY. S.Grooms* and L.S.Jones, Dept. of Developmental Biology & Anatomy, Univ. South Carolina, Sch. of Med., Columbia, SC 29208.

Integrins mediate cell/cell and cell/matrix interactions, and also participate in the transduction of information from the extracellular environment to the intracellular milieu. As many extracellular matrix (ECM) molecules contain the conserved amino acid sequence arg-gly-asp-ser (RGDS) at the integrin recognition site, integrin-ECM binding can be disrupted using RGDS molecules. In this study, we have used the RGDS tetrapeptide to examine a potential role for integrins in an animal model of epileptogenesis termed *in vitro* kindling. 625 μm hippocampal slices were stimulated via the Schaffer collaterals (SCs), and extracellular recordings were made from stratum pyramidale of CA1 and CA3b. All slices were monitored for 15 min via input/output curves in a submersion chamber with constant flow (4-5 ml/min) for 15 min. Slices were then reduced to a recycling submersion chamber (4.5-6 ml/min), monitored for one hour, and then stimulated with a maximum of 6 trains through the SCs delivered at five minute intervals. Experimental groups were washed in 1mM RGDS or the inactive tetrapeptide GGGG for one hour prior to *in vitro* kindling. The control tetrapeptide had no effect on *in vitro* kindling, but in the presence of the RGDS tetrapeptide, there was a statistically significant decrease in the number of stimulus trains required to induce spontaneous bursting. In addition, the RGDS-treated slices displayed a significant increase in the rate of bursts produced spontaneously. The period of spontaneous bursting also increased dramatically in RGDS-treated slices. Our results indicate that in the presence of the competitive peptide, hippocampal slices become hyperexcited over time, indicating that interfering with ECM-integrin binding may alter neuronal stability. Because disruption of the ECM-integrin interaction appears to increase neuronal excitability, these results suggest that integrins may participate in neuronal signaling through an RGDS binding site.

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772.16

ELECTRICAL KINDLING IS ASSOCIATED WITH A LASTING INCREASE IN THE EXTRACELLULAR LEVELS OF KYNURENIC ACID IN THE RAT HIPPOCAMPUS. **[1]R. Schwarcz*, [1]H.-Q. Wu, [2]A. Monno and [2]A. Vezzani, [1]Maryland Psychiatric Research Center, Baltimore, MD 21228 and [2]Mario Negri Institute for Pharmacological Research Laboratory of Neuropharmacology, Milano, Italy.**

The extracellular levels of kynurenic acid (KYNA), an endogenous broad spectrum excitatory amino acid receptor antagonist with anti-neurotoxic and anticonvulsant activity, were assessed by microdialysis in the hippocampus of kindled rats. One week after the completion of amygdala or hippocampal kindling (stage 5: occurrence of tonic-clonic convulsions), the extracellular concentration of KYNA was 1.7 ± 0.1-fold higher than in sham-operated controls ($P < 0.01$). This effect was observed to the same extent in the hippocampus ipsilateral and contralateral to the electrical stimulation. Moreover, whereas 50 μM veratridine, applied through the probe, reduced extracellular KYNA by 28% within 1 h in control rats ($P < 0.05$), veratridine was ineffective in stage 5 kindled rats. During the initial phases of hippocampal kindling (stage 2: stereotypies, retraction of a forelimb), extracellular KYNA levels and the effect of veratridine were similar to controls. The activity of KYNA's biosynthetic enzyme, kynurenine aminotransferase, which is preferentially localized in astroglia, did not change in the hippocampus one week after stage 5 seizures. These data indicate an enhanced liberation of KYNA from hippocampal astrocytes due to an impairment of its normal regulatory mechanisms in fully kindled animals. This may be of relevance for the control of excitatory amino acid receptor function during epileptogenesis.

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772.17

ELECTRICAL STIMULATION OF DISCRETE REGIONS IN THE RODENT AMYGDALA ELICITS DIFFERENCES IN SEIZURE DEVELOPMENT AND RESPONSIVITY. M. Sitcoske, J.B. Rosen, S.R.B. Weiss, R.M. Post* Biological Psychiatry Branch, NIMH, Bethesda, Md. 20892

This study examined whether electrically stimulating different nuclei in the amygdala would produce changes in afterdischarge threshold (AD) and seizure development. Male rats were implanted with electrodes aimed at the basolateral (BL) and central (Ce) nuclei and the amygdalostriatal (AmS) zone. The animals were stimulated once/day with 60Hz, biphasic peak to peak square wave current for 1 sec. The AD threshold values were determined prior to kindling to match and divide the animals into 2 groups. Rats were then kindled at threshold or suprathreshold (800μA) current intensities. Data from 52 animals show that the AD thresholds elicited from the three regions differed from one another prior to kindling ($p<.0001$). The mean AD threshold for the BL nucleus was 74μA; the AmS area was 222μA; and the Ce nucleus was 435μA. Once kindled, AD thresholds were redetermined. Kindling at suprathreshold current resulted in an increase in threshold in the BL and Ce nuclei ($p<0.05$) but not in the AmS zone. These findings are consistent with the earlier work of Pinel and associates (1974), and suggest that kindling at threshold vs suprathreshold current intensities elicits different neurophysiological and biochemical actions. In addition, although the AD thresholds in the Ce nucleus were the highest, this area required fewer number of ADs than the BL nucleus to produce three consecutive stage 5 seizures at either stimulation intensity, suggesting the relationship between threshold and kindling susceptibility is complex.

The BL, AmS, and Ce regions contain cells with distinct morphologies and projection pathways. Although Goddard (1969) originally proposed that stimulation of any region within the amygdala produced relatively similar kindled seizure progression, we and others (Le Gal La Salle, 1982; Gilbert, 1981; Cain, 1992) are finding that differences do exist within the amygdala and delineating these may further our understanding of the anatomical progression of seizure development and its biochemical and behavioral consequences.

772.18

ROLE OF NERVE GROWTH FACTOR IN KINDLING AND KINDLING-INDUCED MOSSY FIBER SPROUTING

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Repeated subconvulsive electrical stimulation of certain areas of the forebrain leads to kindling, a progressive and permanent amplification of evoked epileptiform activity which is a model for human temporal lobe epilepsy. Kindling induces synthesis of nerve growth factor (NGF) protein and mRNA. In addition, mossy fiber sprouting and functional synaptogenesis have been observed in the hippocampus of kindled animals. We have shown that intraventricular administration of antibodies and peptide antagonists of NGF retard amygdaloid kindling and block kindling-induced mossy fiber sprouting. To determine whether NGF can *enhance* the development of kindling and kindling-associated sprouting, rats receiving intraventricular NGF or saline were kindled in the amygdala by twice-daily stimulations. Rats receiving intraventricular NGF required fewer (6.7± 0.9) stimulations to reach the fully kindled state than rats receiving saline (13.7 ± 0.9). Following kindling, sprouting of the mossy fibers into the stratum oriens of the CA3 region of the hippocampus is measured by Timm staining, and kindling-induced hippocampal damage is assessed by performing total cell counts for the hilus. Timm staining and cell counting analyses are in progress and preliminary data will be reported. Our findings further demonstrate the role of NGF in kindling.

772.19

EFFECT OF KINDLING ON CALCIUM CURRENTS IN ADRENALECTOMIZED AND ADRENALLY INTACT RATS. H. Karst[1], A.B. Mulder[2]*, E.R. De Kloet[1] and M. Joëls[2], [1]Med. Pharmacol.,Univ. of Leiden, 2300 RA Leiden; [2]Dept. Exp. Zoology, Univ. of Amsterdam, 1098 SM Amsterdam, The Netherlands.

Stress is known to affect the incidence and nature of seizures in epileptic patients.To study the effect of corticosteroid hormones on epilepsy we used an animal model for temporal lobe epilepsy, i.e. kindling. Via electrodes implanted in the hippocampal Schaffer collaterals, male Wistar rats were tetanized twice daily with 50 Hz stimulation frequency for two seconds with a current intensity of 300 µA. After the tenth class 5 seizure according to the scale of Racine, the rats were fully kindled. Three to four weeks later rats were either adrenalectomized or sham operated. Five to seven days later the animals were sacrificed. Four experimental groups were investigated: non-kindled sham operated, kindled sham operated, non-kindled ADX and kindled ADX rats. Of these groups a number of parameters were studied, including the properties of voltage-gated calcium currents in CA1 neurons, with the in-situ patch-clamp technique in the slice. Kindling and ADX increased the amplitude of low threshold calcium (LTC) currents. Voltage properties and kinetics of (in)activation were not affected. Adrenalectomy of the kindled rats however did not further increase the LTC currents, indicating that ADX is not acting synergistically. In contrast to the LTC-currents, kindling caused a decrease of the high threshold calcium (HTC) currents. Adrenalectomy did not affect these HTC-currents, neither in the non-kindled nor in the kindled rats. The expression of mRNA for voltage gated Ca-channel subunits and corticosteroid receptors in the kindled and ADX rats is presently under investigation. Previously we reported that LTC-channels are mainly located in the dendrites of pyramidal CA1 neurons; HTC-channels can be found near the soma. We tentatively conclude that both kindling and ADX particularly increase calcium influx in the dendritic region, an area where also a considerable part of the synaptically induced Ca-influx takes place.

773.1

CORRELATION BETWEEN THE EXPRESSION OF NICOTINIC ACETYLCHOLINE RECEPTOR α4-SUBUNIT mRNA AND TAU-PROTEIN IN THE CORTEX OF ALZHEIMER PATIENTS. A. Wevers[1*], C. Lobron[2], M. Ghobrial[3], E. Giacobini[4], P. Gass[5], A. Maelicke[2] and H. Schröder[1]. [1]Inst. II für Anatomie, Univ. zu Köln, 50931 Köln, FRG, [2]Inst. für Physiol. Chemie und Pathobiochemie, Univ. Mainz, 55128 Mainz, FRG, Depts. of [3]Pathology and [4]Pharmacology, Southern Illinois Univ. Sch. Med., Springfield, IL, USA, [5]Inst. für Neuropathologie, Univ. Heidelberg, 69120 Heidelberg, FRG

Changes in the expression of nicotinic acetylcholine receptor (nAChR) have been among the first reported neurochemical landmarks of Alzheimer's disease (AD). The availability of nAChR subunit specific nucleic acid probes has allowed to investigate the subunit gene expression and its relation to pathological intracellular changes.

In situ hybridizations were performed on autopsy tissue of the superior frontal gyrus of AD brains (n=6) and age-matched controls (n=5), using a digoxigenin-labeled α4 nAChR subunit-specific riboprobe. Hybrids were visualized by an alkaline phosphatase-coupled digoxigenin antibody. Simultaneous detection of tau-protein or glial fibrillary acidic protein was achieved by subsequent indirect immunoperoxidase technique. As reported earlier, α4 mRNA was detected in many neurons of all cortical layers. We did not observe general changes in the distribution of α4 subunit nAChR expressing neurons in AD as compared to controls. Corresponding to the density of structures expressing tau protein, the number of α4 mRNA-containing apical dendrites was decreased in AD, especially in the pyramidal cells of layer II/III. Neurons that were heavily labeled for the tau-protein expressed little as no α4 mRNA.

The present results underline the importance of the relation between the expression of pathologically modified filaments and nAChR mRNA. Further studies at the EM level on the mRNA distribution of α4 and other subunits will provide additional information on nAChR mRNA transport under normal and pathological conditions.

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773.2

HCNP gene expression is decreased in the hippocampus of postmortem human brain from patients with senile dementia of the Alzheimer type.

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We previously demonstrated that Hippocampal Cholinergic Neurostimulating Peptide (HCNP), a novel peptide purified from 10-12 day old rat hippocampus, specifically enhances acetylcholine synthesis in medial septal nuclei in vitro.The septo-hippocampal system plays an important structural role in memory formation. Moreover, it is well known that certain pathological changes, such as neuron loss, gliosis, plaques and tangles, are found in the hippocampus of patients with Alzheimer type dementia.In this study, we prepared total RNA from hippocampus and frontal lobe of postmortem human brain by the AGCP method. mRNA was quantified by Northern blot analysis using cDNA specific for HCNP precursor protein as a probe.The results suggested that expression of HCNP precursor mRNA in hippocampus decreased in 5 cases with Alzheimer type dementia compared with 5 cases of normal aging, even after correction for the expression of β-actin mRNA.This result surpports our previous findings documenting the lack of neurotrophic substances in brains of patients with Alzheimer's disease. (Ojika,K. et.al., Banbury report 15,Cold spring Harbor,NY.1983;285-295).

773.3

FUNCTIONAL REGULATION OF NEOCORTICAL PYRAMIDAL NEURONES BY BOTH MUSCARINIC AND NICOTINIC RECEPTORS. I.P.Chessell, M.A. Simmonds* and P.P.A.Humphrey Glaxo Institute of Applied Pharmacology, Cambridge, CB2 5DH, and *Dept. Pharmacology, School of Pharmacy, London, WC1N 1AX, UK.

A novel cortical brain slice preparation for use with extracellular "grease-gap" recording techniques has been developed, allowing study of both glutamatergic and cholinergic responses of neocortical pyramidal neurones which form the transcallosal-corticocortical pathway. Concentration-effect curves to the muscarinic/nicotinic agonist carbachol and the nicotinic agonist DMPP yielded mean EC_{50} values of 29.5 and 13.2 μM, respectively. Carbachol-induced responses were inhibited by atropine (0.2 μM) and mecamylamine (10 μM). This antagonism was not competitive. Conversely, concentration-effect curves to DMPP were shifted in a parallel rightward manner by mecamylamine, and were unaffected by atropine. Depolarisation responses to glutamate were reversibly antagonised by D-AP5 (30μM) and CNQX (15 μM); these antagonists had no effect on carbachol- or DMPP-induced responses.

This preparation allows reproducible quantification of depolarisation responses of pyramidal neurones whose axons pass through the corpus callosum to contralateral cortex; such studies indicate functional regulation of the activity of these neurones by nicotinic and muscarinic receptors present on their cell bodies and dendrites. These same neurones degenerate in Alzheimer's disease, culminating in marked cortical glutamatergic hypoactivity, which underlies the cognitive deficits observed. We propose, therefore, that therapies aimed at activating nicotinic as well as muscarinic receptors are more likely to succeed in ameliorating the cognitive symptoms of the disease than those aimed at muscarinic activation alone.

773.4

DECREASED BINDING OF THE VESAMICOL ANALOG [^{125}I]-(+)-MIBT TO THE TEMPORAL CORTEX IN ALZHEIMER'S DISEASE. E.M. Garland, I.K. Staley, D.C. Mash, M. Basile* and S.M.N. Efange. Depts. of Neurology & Moll. Cell. Pharmacology, Univ. of Miami School of Medicine, Miami, FL. 33101; Depts. of Radiology, Med. Chem. & Neurosurgery, Univ. of Minnesota, Minneapolis, MN 55455.

The vesamicol binding site on the cholinergic synaptic vesicle is a novel target for development of radiopharmaceuticals for mapping cholinergic innervation. Previous studies using [^{3}H]vesamicol have demonstrated marginal reductions in the densities of vesamicol binding sites in frontal cortex and increased densities in the temporal cortex in Alzheimer's disease (AD). Vesamicol binding site densities correlated poorly with the loss of choline acetyltransferase (ChAT) activity in AD. These conflicting findings suggest that [^{3}H]vesamicol may label also non-cholinergic sites in the cerebral cortex. In the present study, we have evaluated the novel vesamicol analog [^{125}I]-(+)-MIBT as a probe to assess cholinergic synaptic integrity in the temporal cortex of AD and neurologically-normal age matched control subjects. Saturation binding analysis using aminobenzovesamicol (ABV) to define non-specific binding, revealed a high affinity binding site with a K_D value of 3.2 ± 0.2 nM in the temporal cortex in aged control subjects. Similar affinity values were observed for [^{125}I]-(+)-MIBT binding in AD. The density of [^{125}I]-(+)-MIBT binding was reduced significantly in AD temporal cortex (Bmax =11.6 ± 2.8 pmol/g) as compared to control values (Bmax =19.5 ± 3.2 pmol/g). The decrease in [^{125}I]-(+)-MIBT binding was correlated with ChAT activities (r = 0.90) in AD temporal cortex. These results suggest that [^{123}I]-(+)-MIBT may be useful for assessing *in vivo* the loss of cholinergic projections with SPECT in AD and other dementing disorders.

773.5

SELECTIVE ACETYLCHOLINE NEUROTOXINS, IN COMBINATION WITH GLUCOCORTICOIDS AS A POTENTIAL MODEL OF ALZHEIMER'S DISEASE: THE EFFECTS OF AF64A AND SAPORIN ON SPATIAL LEARNING IN THE MALE RAT. W.A. Dornan*, A.R.V. McCampbell, G.P.Tinkler, L.J. Hickman, Dept. of Psych., Illinois Wesleyan Univ., Bloomington, IL. 61702.

Alzheimer's disease currently afflicts approximately 4 million people in the United States, with 100,000 new cases being reported each year. This disorder is typified by several cognitive deficits, including memory loss. In our laboratory we have taken several approaches to the generation of a suitable animal model with which to study this disease. Previous work has focused on exploring a possible synergistic effect between a neurotoxic protein (beta amyloid) found in AD patients and stress. In the following series of studies we have expanded on these results in glucocorticoid-treated animals following ICV injections of the ACh neurotoxin, AF64A (1nmol/ventricle), or intracerebral injections of the ACh toxin, saporin (.42ug). In experiment 1, four groups of male rats were used: 1) ICV injections of AF64A in animals treated for 7 days with corticosterone (CORT,7 mg), 2) ICV injection of vehicle + CORT, 3) ICV injections of AF64A + 7 days of sesame oil (CONT), and 4) ICV injections of vehicle + CONT. In experiment 2 the following groups were used: 1) bilateral injections of saporin into the nucleus basalis (NB), 2) bilateral injections of vehicle into the NB (CONT), 3) unilateral injections of saporin into the medial septal area (MSA), and 4) unilateral injections of vehicle into the MSA. Approximately 14 days following surgery, all animals were assessed for spatial learning using a modified version of the Morris Water Maze. Our results reveal a significant impairment on a variety of behavioral parameters associated with spatial learning in the rat following injections of saporin into the NB and the MSA. In support of previous research, a significant recovery was observed in the MSA animals. In addition, a significant potentiation of AF64A on spatial learning was observed in glucocorticoid-treated animals. Our results strongly support the use of selective ACh neurotoxins as a viable model of Alzheimer's disease, and further suggest that chronic stress is involved in the pathogenesis of Alzheimer's disease.

773.6

SCOPOLAMINE-INDUCED IMPAIRMENT OF CONTINUOUS PERFORMANCE IN RHESUS MONKEYS IS REVERSED BY TACRINE (COGNEX) AND THE m_1 SELECTIVE MUSCARINIC AGONIST PD 151832. M. J. Callahan*. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48105.

Pharmacologic blockade of postsynaptic muscarinic receptors impairs cognitive function in animals and man. The muscarinic receptor antagonist scopolamine impairs various aspects of memory including deficits in selective attention. The role of an intrinsic attentional deficit in Alzheimer's disease and the muscarinic cholinergic contribution to these deficits may be important in developing appropriate therapy.

In this study, test experienced rhesus monkeys with established baseline performance were used. Responses on a continuous performance task were measured using a microcomputer-controlled test environment. Animals were rewarded for responding to a target object displayed on the screen of a touch-sensitive color television monitor (CRT). Display of the target object on the CRT was randomized with respect to time and spatial location. Scopolamine (0.003, 0.006 and 0.01 mg/kg, IM) produced a dose-dependent decrease in responses which was seen to persist 2 hrs after dosing. Tacrine (0.03, 0.1, 0.32 and 1.0 mg/kg, IM) given 60 min after scopolamine and 30 min before testing reversed the impaired performance produced by scopolamine in a dose-dependent manner. PD 151832 (0.1, 0.32 and 0.1 mg/kg, IM) given similarly also improved performance in a dose-dependent manner. These data suggest that scopolamine produces a deficit in sustained attention or slowing of information processing that can be attenuated by treatment with tacrine or PD 151832.

773.7

MUSCARINIC AGONISTS INCREASE HIPPOCAMPAL PHOSPHATIDYL INOSITOL TURNOVER *IN VIVO* AND ATTENUATE HEMICHOLINIUM-3-INDUCED AMNESIA IN MICE. K.M. Ward, D.S. Chapin, J.T. Nowakowski, J.T. Forman, D.M. Nason, A. Villalobos and D.R. Liston*, Pfizer Inc., Central Research Division, Deptartment of Neuroscience, Groton, CT 06340.

Alzheimer's disease (AD) results in a loss of the cholinergic projections important in learning and memory. A muscarinic agonist that interacts directly with post-synaptic m1 receptors, which are preserved in AD, may provide effective palliative therapy. We have examined the *in vivo* activity of three potent muscarinic agonists (pilocarpine, LY-246708 and SDZ-ENS-163) using biochemical (*in vivo* phosphatidyl inositol turnover) and behavioral (passive avoidance) models of central muscarinic activation. For *in vivo* PI turnover, ^{3}H-myoinositol was injected i.c.v. (2 µCi/5 µL) 24 hr prior to test compounds to prelabel the hippocampal phospholipid pool. Lithium (10 meq/kg s.c.) was administered 3 hr prior to tissue sampling and the test compounds were administered s.c. 1 hr prior to tissue sampling. The accumulation of inositol phosphates (IP_3) in the hippocampus was determined following extraction and ion exchange chromatography. Significant elevation of hippocampal IP_3 was produced by pilocarpine at 10-32 mg/kg s.c.; by LY-246708 at 3-45 mg/kg s.c.; and by SDZ-ENS-163 at 5-25 mg/kg s.c. A one-trial step-through passive avoidance (PA) procedure was utilized to test the ability of these 3 compounds to reverse hemicholinium-3 (HC-3) induced amnesia. Test step-through latency was reduced by 50-60% following treatment with HC-3 (1.78 µg i.c.v.) two hours prior to the training session. Pilocarpine (1.78-10 mg/kg), LY-246708 (1-10 mg/kg) and SDZ-ENS-163 (1.78-17.8 mg/kg) attenuated HC-3-induced amnesia. Most notably, the effective dose range of LY-246708 and SDZ-ENS-163 in PA spanned a 10-fold range of doses, in contrast to our experience with AChE inhibitors, which show a more narrow range of effective doses in this model. We conclude that selective muscarinic agonists may be useful in treating disease states such as AD where the central cholinergic system is compromised.

773.8

COMBINED CHOLINERGIC AND ADRENERGIC CANDIDATES FOR ALZHEIMER'S DISEASE (AD) THERAPY (I): PHARMACOLOGICAL CHARACTERIZATION. H.M. Vargas*, G. Bores, W. Petko, M. Li, D. Selk, D.K. Rush, L. Davis, D. Fink and C.P. Smith. **Hoechst-Roussel Pharmaceuticals, Inc., Neuroscience PGU, Somerville, New Jersey 08876.**

The most widely characterized neurotransmitter deficiencies in AD are of the cholinergic and noradrenergic systems. Clinical studies with acetylcholinesterase inhibitors (AChEI) have shown some symptomatic improvement, but the efficacy of these agents is low. Behavioral literature suggests that norepinephrine (NE) enhances learning and memory in several different animal models. Based on these findings, we developed novel AChE inhibitors which also possess noradrenergic properties. For these studies, all *in vitro* biochemical indicies were derived from rat and dog tissues. In rat brain, the following activities were determined: potency at inhibiting AChE activity, α_2-adrenoceptor affinity as determined by [^{3}H]clonidine displacement, and the functional release of [^{3}H]NE due to presynaptic α_2-adrenoceptor blockade. Antagonism of BHT-920 induced contractions of the dog saphenous vein was used to functionally determine the nature of α_2-adrenoceptor blockade *in vitro*. Our findings indicate that compounds P11467 and P11345 demonstrated pharmacological activity as combined AChE inhibitors with competitive α_2-adrenoceptor antagonist properties. These compounds were inactive as inhibitors of norepinephrine, dopamine and serotonin uptake. In addition, these compounds were inactive as monoamine oxidase inhibitors, nor did they have affinity for muscarinic and dopaminergic receptors. These agents are further discussed in this and accompanying abstracts.

773.9

COMBINED CHOLINOMIMETIC AND ADRENERGIC CANDIDATES FOR ALZHEIMER'S DISEASE (AD) THERAPY (II): *IN VIVO* BIOCHEMICAL ACTIVITY. A. Giovanni*, G. Bores, M. Li, C.P. Smith, L. Zhou, D. Cunningham and H.M. Vargas. **Hoechst-Roussel Pharmaceuticals, Inc., Neuroscience PGU, Somerville, New Jersey 08876.**

Cholinergic deficits appear to correlate with the severity of memory impairment associated with AD. Acetylcholinesterase inhibitors (AChEI) have shown symptomatic improvement although deficits in other neurotransmitters, e.g. norepinephrine (NE), have been implicated in AD. Camacho *et al.* (1995; next abstract) have shown α_2-antagonists potentiate the ability of AChEI's to enhance long-term memory retention. We describe studies in which we examine several *in vivo* parameters to assess the efficacy of a novel AChEI with noradrenergic properties (prior abstract, Vargas *et al.*, 1995). P11467 dose-dependently inhibited rat striatal AChE activity (ID_{50}=3.1 mg/kg, i.p.); this effect was reversible. In microdialysis studies, the compound increased ACh levels in the rat hippocampus 4-fold following a dose of 5 mg/kg i.p., an effect shared with other AChEIs. At 1.25 - 5 mg/kg, i.p., P11467 significantly increased hippocampal NE release (similarly to idazoxan) in a dose-dependent manner, a characteristic not shared with other AChEIs. Doses of P11467 which elevated ACh and NE release also enhanced long-term memory retention in a step-down passive avoidance paradigm in rats. Functional assays showed that P11467 competitively antagonized pre- and postsynaptic α_2-adrenoceptors *in vitro* and *in vivo*. These findings indicate that P11467 elevates central ACh and NE levels via inhibition of AChE activity and antagonism of α_2-adrenoceptor function.

773.10

COMBINED CHOLINERGIC AND ADRENERGIC CANDIDATES FOR ALZHEIMER'S DISEASE (AD) THERAPY (III): *IN VIVO* EFFICACY. F. Camacho, H.M. Vargas, C.P. Smith, J.T. Winslow.* **Hoechst-Roussel Pharmaceuticals Inc., Neuroscience Product Group, Somerville, N.J., 08876**

The cholinergic hypothesis of AD has influenced much of research on learning and memory over the last decade. However, cholinomimetic agents have had limited success in the treatment of AD dementia. The noradrenergic system has also been implicated in cognitive processes and is depleted in AD. This study investigated the effects of enhanced noradrenergic and cholinergic neurotransmission on cognitive measures in rodents. Acetylcholinesterase inhibitors (AChEI) at moderate doses significantly enhanced retention latencies in a step-down passive avoidance (EPA) paradigm. The α_2-adrenoceptor antagonists idazoxan (IDA), yohimbine (YOH), and P7480 had no effect on the retention response in EPA. However, when a sub-threshold dose of heptylphysostigmine (HEP) was administered in combination with IDA, YOH, or P7480 there was a potentiating effect that led to an enhanced retention of the passive avoidance response. This enhanced retention was not evident when HEP was coadministered with either the systemically active, but not centrally active, α_2-adrenoceptor antagonist MK-467 or the postsynaptic α_2-adrenoceptor antagonist SKF 108456. The data indicate that central cholinergic and noradrenergic systems may interact in the formation of a long-term memory trace. Mixed AChEI-α_2-adrenoceptor antagonist compounds P11467 and P11345 were also tested in the EPA and were found to significantly enhance the retention of the passive avoidance response in rats. These results suggest that drugs which enhance cholinergic and noradrenergic activity may be an efficacious in the treatment of AD.

773.11

CHANGES IN BRAIN ACETYLCHOLINE OVERFLOW DURING RAT AND MONKEY MICRODIALYSIS FOLLOWING ADMINISTRATION OF THE ACETYLCHOLINESTERASE INHIBITOR, CI-1002. M. Duff Davis* and Leonard W. Cooke, Neurosciences Therapeutics, Parke-Davis Research, Division of Warner-Lambert, 2800 Plymouth Rd., Ann Arbor, MI 48105.

Pathological findings have indicated that cholinergic innervation of the brain in Alzheimer's patients appears to be compromised. In the clinic, some patients have shown improvement or delayed progression of symptoms during treatment with acetylcholinesterase (AChE) inhibitors such as tacrine. Here we report on a new AChE inhibitor and muscarinic antagonist, CI-1002, and its ability to alter neurotransmitter release in two animal species.

Adult male Long-Evans rats had microdialysis probes surgically implanted into the lateral prefrontal cortex and were allowed to recover for 24hrs. Male squirrel monkeys, Saimiri Sciureus, were chair-restrained and a probe placed in the putamen through a previously-implanted guide cannula. CI-1002 was administered at 0.32, 1.0 or 3 mg/kg im with each dose evoking an increase in basal ACH overflow compared to control. This study demonstrates the potential of CI-1002 to elevate cholinergic tone in rodent and non-human primate species.

773.12

DETERMINATION OF BLOOD AND BRAIN ACETYLCHOLINESTERASE (AChE) INHIBITION AFTER ORAL DOSING OF CI-1002. M.R. Emmerling, M.C. Callahan, H. LeVine*, W. Lipinski, & C. Raby. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert, 2800 Plymouth Rd., Ann Arbor, MI 48105.

CI-1002 is a combined AChE inhibitor and muscarinic antagonist (Mol. Neurobiol., 1994, 9, 93). AChE enzyme inhibition in blood and different brain regions produced after oral dosing of 2 and 10 mg/kg doses of CI-1002 was determined by ex vivo radiometric assay. AChE activity in blood after the 2 mg/kg dose was decreased by 40 % compared to control at 0.5 hr and gradually returned to control levels by 4 hours. The 10 mg/kg dose reduced AChE activity in blood by 80% compared to control at 0.5 hr. AChE activity rose over time but remained significantly lower than control over the next 6 hr. In brain, the enzyme activity in hippocampus was significantly reduced by 30% at 1.0 hr at the 2 mg/kg dose of CI-1002 and by 54% at 0.5hr at the 10 mg/kg dose. AChE activity at the 10 mg/kg dose remained significantly less than control for the next 2 hr. The time course of AChE inhibition in hippocampus was similar to that seen in blood at both doses of CI-1002. Significant inhibition of AChE activity was also evident in the frontal cortex at 1 and 2 hr after the 10 mg/kg dose. Although lower levels of AChE activity were detected, no significant changes in the AChE activity of striatum or cerebellum relative to control was detected at any dose or time point tested. These results show that CI-1002 enters the brain in sufficient quantities to cause inhibition of AChE activity. The level of AChE inhibition in brain is dose-dependent and correlates with the level of AChE inhibition detected in the blood. Brain regions are differenetially affected by CI-1002 with the greatest inhibition being detected in the hippocampus. Finally, results will be presented to show that the level of AChE inhibition detected can be used to estimate the concentration of CI-1002 in tissues with reasonable accuracy.

773.13

BUTYRYLCHOLINESTERASE IMMUNOCHEMISTRY IN HUMAN CEREBRAL CORTEX. M.-M. Mesulam*[1], C. Geula[2], S. Brimijoin[3], J.F. Smiley[1]. [1]Northwestern University, Chicago IL; [2]Harvard Medical School, Boston MA; [3]Mayo Foundation, Rochester MN.

Plaques and tangles in Alzheimer's disease (AD) express butyrylcholinestrase (BChE) enzyme activity. This activity could conceivably come from degenerated remnants of premorbidly BCHE-positive neurons. To test this hypothesis, we used monoclonal antibodies to detect BChE- immunopositive neurons in the human cerebral cortex. There were very few BChE-positive perikarya in non-demented young and aged brains. Most were located in the gyral white matter or in layer 6 and were non-pyramidal. Limbic areas, especially the stratum oriens of the hippocampus, contained the highest number. The density of these neurons was 1-2 orders of magnitude less than that of BChE-positive tangles seen in AD. No immunopositive cortical axons were seen to account for the BChE-positivity of neuropil plaques in AD. Concurrent visualization of BChE immunoreactivity with BChE enzyme activity revealed distinctly double-labeled perikarya.

These results support our conclusion that the plaque- and tangle-bound BChE in AD is unlikely to be of neuronal origin. In many of our specimens, neuroglia appeared intensely BChE-immunopositive. Since neuroglia are prone to non-specific immunostaining, this observation needs to be interpreted cautiously. However, the BChE-immunopositivity of neuroglia is consistent with our histochemical experiments and our hypothesis that the plaque- and tangle-bound BChE is of neuroglial rather than neuronal origin. The role of neuronal BChE remains mysterious. Diverse trophic functions have been attributed to BChE and may underlie its role in neuroglia.

773.14

EFFECT OF THE SELECTIVE, REVERSIBLE ACETYLCHOLINESTERASE INHIBITOR E2020, QUANTIFIED BY DFP ANTAGONISM. K. A. Sherman*. Dept. Pharmacol. & Therap., Univ. So. Florida Coll. Med., Tampa, FL 33647

E2020, a piperidine derivative, is a specific, CNS-active, reversible inhibitor of acetylcholinesterase (AChE) that appears to be well-tolerated in humans and animals. Because the drug acts by reversible binding to AChE, its inhibitory effect is reversed by tissue dilution and consequent drug dissociation. Therefore we developed an alternative approach for quantifying E2020's effect on AChE after *in vivo* administration -- measuring the ability of the reversible drug to antagonize diisopropylfluorophosphate (DFP), a hemi-substrate of AChE that produces irreversible phosphorylation, while reacting slowly enough for rate determination at minimal tissue dilution. In this study, we determined the effect of two oral doses of E2020, 5 and 8 mg per day, administered to young male human subjects for up to 14 days, on red blood cell (RBC) AChE by the DFP antagonism technique. Measured in RBC collected 4 hr after drug, at the time of peak plasma concentrations, the effect of 5 mg E2020 on AChE was already nearly maximal by 3 days ($\bar{X} \pm$ S.E.M. at 7 d: 38 ± 1.3%). The degree of DFP antagonism was dose-related. DFP phosphorylation was inhibited nearly 60% after 14 days on the 8 mg dose ($\bar{X}$ ± S.E.M.: 59 ± 1.1%). The effect on RBC AChE was substantially less 72 hr after the last dose, but more than half-maximal effect persisted still in most subjects. As shown in dementia patients treated with 1-2 mg E2020 daily, the degree of DFP antagonism was linearly related to the log of plasma drug concentration; the IC_{50} plasma concentration is about 40 ng/ml E2020. Plasma ChE was unaffected. In companion rat studies, we report that the threshold dose for elevating extracellular ACh in cortex, 0.5 mg/kg, produces about 50% inhibition of DFP phosphorylation at 30 min. Following 2 mg/kg E2020, a dose that elevates microdialysate ACh 20-fold, DFP phosphorylation of cortical AChE is completely blocked at 30 min. Thus, doses of E2020 which are well tolerated in humans produce an effect on AChE of magnitude sufficient to enhance brain cholinergic function.

773.15

PHENSERINE, A NEW DRUG FOR ALZHEIMER'S DISEASE: FAVORABLE TOXICITY PROFILE.

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Phenserine, a phenylcarbamate of (-)-physostigmine, is a novel, long acting ($t_{1/2}$=8 h), rapidly cleared ($t_{1/2}$=10 min), brain-targeted (10-fold), and acetyl- selective (>50 fold) cholinesterase inhibitor. In rats, it significantly increases brain acetylcholine and robustly enhances cognition [NeuroReport 6: 481-4, 1995; Med. Res. Rev. 15: 3-31, 1995].

Phenserine is dramatically less toxic than physostigmine (MTD >15 vs. 0.6 mg/kg, ip, rat). In initial toxicological studies, phenserine was administered once daily for 28 consecutive days to rats at doses of 1 and 5 mg/kg ip (cumulative doses of 28 and 140 mg/kg) and was compared to saline-treated animals. Extensive evaluation of blood hematologic and chemical parameters, including markers of renal and hepatic function, together with histological analysis of brain, kidney and liver, demonstrated lack of toxicity at doses more than five times greater than those which maximally enhance cognition. A standard pharmacologic screen revealed no evidence of direct action at neurotransmitter receptors and lack of autonomic activity in mice.

Phenserine's optimal pharmacological properties, combined with its favorable toxicological profile in pre-IND studies, predict efficacy in upcoming clinical trials superior to that of other agents developed for Alzheimer's disease.

773.16

EFFECTS OF THE ACETYLCHOLINE RELEASER LINOPIRDINE ON SPECT RCBF AND COGNITIVE FUNCTION IN ALZHEIMER'S DISEASE. C. H. van Dyck*, C. H. Lin, R. Robinson, J. Cellar, M. Narayan, A.F.T. Arnsten, and P.B. Hoffer. Yale University School of Medicine, New Haven, CT, 06520.

Centrally acting cholinergic drugs have been reported to increase regional cerebral blood flow (rCBF) as measured by single photon emission computed tomography (SPECT) in brain regions affected by Alzheimer's disease (AD). We studied the effects of the acetylcholine releaser linopirdine (LPD) on SPECT rCBF and on cognitive, behavioral, and global variables in patients with probable AD. Twenty-four AD patients (12 M, 12 F; mean age ± SD = 68.9 ± 8.2 years) and 13 healthy controls (8 M, 5 F; 68.4 ± 8.0 years) participated. AD patients were scanned with 20 mCi of Tc-99m-ECD using the CERASPECT at baseline and following 4 weeks of treatment with linopirdine 40 mg TID (n=15) or placebo TID (n=9) in a double-blind trial. Healthy subjects were scanned for comparison with baseline AD scans. A ratio of parietal/cerebellar rCBF (P/C) was derived. The effect of diagnostic group (AD vs. healthy) on P/C was determined by t-test. The effect of treatment condition on P/C and behavioral measures in the AD patients was subsequently examined by t-tests. Significant differences in P/C were found between AD patients (0.70 ± 0.09) and controls (0.88 ± 0.08) ($p<.0000001$). Patients treated with LPD showed an increase in P/C of 4.2% ± 6.0%; whereas those treated with placebo showed a decrease of -1.2% ± 7.5% ($t=2.31$; $df=22$; $p=.03$). Active treatment was also associated with improvement in the Clinical Global Impression of Change ($t=2.14$; $p=.04$) and trends toward improvement in the Alzheimer's Disease Assessment Scale ($t=1.89$; $p=.07$) and the Dementia Behavior Disturbance Scale ($t=1.91$; $p=.07$). These data support the conclusion that rCBF abnormalities in AD are, in part, truly "functional" and can be selectively altered with pharmacological interventions.

773.17

APOLIPOPROTEIN E4 AND CHOLINERGIC DYSFUNCTION IN ALZHEIMER DISEASE: EFFFECT ON TREATMENT OUTCOME. M.-C. Delisle*, J. Poirier, R. Quirion, I. Aubert, M. Farlow, P. Bertrand, D. Dea, S. Gauthier, J. Nalbantoglu and B.G. Gilfix. Douglas Hospital Research Centre, Neuroscience Division, 6875 LaSalle Blvd, Verdun, Québec, Canada. H4H 1R3.

Apolipoprotein E (apoE) is implicated in cholesterol and phospholipid transport, particularly in the central nervous system where other apolipoproteins such as apoA1 and apoB are absent. ApoE is a polymorphic protein of which the major allelic forms are apoE2, apoE3 and apoE4. The latter is associated with familial and sporadic forms of Alzheimer's disease (AD). The number of apoE4 gene copies affects the age of onset, the accumulation of senile plaques and choline acetyltransferase (ChAT) activity in the hippocampus of AD patients (Poirier *et al.*, Lancet, 1993, 342:697-9; Schmechel *et al.*, 1993, PNAS 90:9649-53; Poirier, 1994, TINS 17:525-9). To further characterize the impact of the apoE4 allele(s) on cholinergic deficits in AD, we examined the effect of the number of apoE4 allele copy number on residual ChAT activity, and nicotinic and muscarinic M1 and M2 receptor sub-types in the hippocampus and temporal cortex of post-mortem brains from AD and control patients with different apoE genotypes. The apoE4 allele copy number shows an inverse relationship with residual ChAT and nicotinic binding sites in AD. Such a relationship was not seen in control patients. AD individuals lacking the apoE4 allele show a residual ChAT activity and nicotinic receptor levels rather similar to control values. ApoE4 allele copy number did not correlate with apparent changes in M1 or M2 binding sites in AD. Finally, the effect of apoE4 allele copy number on cholinomimetic drug response was examined in AD patients treated for six months with the esterase inhibitor, Tacrine. Data revealed that over 80% of the patients who do not possess the ApoE4 allele showed significant improvements following this therapy while 60% of the patients with at least one copy of the apoE4 gene did not improve, and even deteriorated, during the treatment These results strongly suggest that apoE4 plays an active role in cholinergic dysfunctions associated with AD. Supported by MRCC.

774.1

SIGNIFICANT CHANGES IN THE HUMAN BRAIN HISTAMINERGIC SYSTEM IN ALZHEIMER'S DISEASE.

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Histamine is one of the neuromodulators present in subcortical projection systems. Neurofibrillary tangles are present in histaminergic neurons in Alzheimers disease, but the target areas have not been examined. We combined sensitive immunohistochemical methods with HPLC fluorometry to reveal the changes in brain histamine in Alzheimer's disease.

In normal human brain (n=5, mean age 82 yrs), histamine concentrations were highest in the hypothalamus, followed by substantia nigra, putamen, n. caudatus and different cortical areas. In Alzheimer's disease (n=9, mean age 81.5 yrs) histamine content was significantly reduced only in the hypothalamus (57%), temporal cortex (46%) and hippocampus (56%). Immunohistochemistry revealed only neuronal storage sites of histamine in normal brain and in brain affected by Alzheimer's disease. In normal brains, dense networks of histamine-containing fibers were seen in the entorhinal cortex and subiculum. Moderately dense networks innervated hippocampal fields CA1-4 and dentate gyrus. Long fibers were seen in the fimbriae. The results reveal an extensive histaminergic system in human temporal lobe, and significant changes in this system in Alzheimers disease.

774.2

NEUROFIBRILLARY TANGLES AND TYROSINE HYDOXYLASE IMMUNOREACTIVITY IN THE LOCUS COERULEUS IN ALZHEIMER'S DISEASE.

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The neurofibrillary tangle (NFT) of Alzheimer's disease is composed of insoluble tau isoforms containing multiple phosphorylations. Currently, it is unknown whether the phosphorylation(s) of tau is causal in the aggregation and accumulation of the NFT. However, a phosphorylation at serine 199/202 of tau occurs in the soma and processes of neurons in the absence of detectable NFT. This phosphorylation, identified using monoclonal antibody AT8, also appears to be contained within the NFT proper. Thus, cellular distribution of AT8 staining identifies neurons from a presumptive early stage through the progressive accumulation of pathology within the neurites and neuronal soma.

We hypothesize that as the intracellular NFT accumulates, neuronal function declines. Specifically, as pathology accumulates within the neurites and eventually the cell body, neurotransmitter synthesis declines prior to cell death. The goal of this study is to determine the neurotransmitter synthetic capacity within individual noradrenergic neurons in the locus coeruleus of Alzheimer's' patients as a function of NFT accumulation.

In this study double labeling ICC was performed using antibodies directed against tyrosine hydroxylase (TH; Chemicon Int., Inc.) and phosphorylated serine 199/202 of tau (AT8; Innogenetics Inc.) within the locus coeruleus of AD patients. The degree of intracellular tau pathology in neurons immunoreactive for AT8 was determined according to the criterion of Braak et al., (1994). Noradrenergic neurons were analyzed for tau pathology and TH immunoreactivity was classified as strong, moderate or weak based on staining intensity. Neuromelanin also served to identify noradrenergic neurons containing NFT in the absence of TH immunoreactivity. Preliminary results indicate that as tau pathology accumulates, TH immunoreactivity declines prior to cell death. Supported by grants: Training Grant 5-T32 AG00107-12, ADC 5-P30 AG08665, LEAD 5-R35 AG09016, R01 AG1121-14.

774.3

EFFECTS OF THE RELEASE ENHANCER DuP 996 ON THE IN VIVO RELEASE OF DA AND 5-HT IN RAT STRIATUM.

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DuP 996 (Linopirdine) is a release enhancer which possibly acts via interaction with a K-channel. It is now well established that *in vitro*, DuP 996 increases the K^+ stimulated release of ACh, DA and 5-HT from slices, while microdialysis studies have shown that DuP 996 also inreases *in vivo* ACh release. To determine the effects of DuP 996 on *in vivo* DA and 5-HT release, we measured extracellular DA and 5-HT levels by microdialysis in rat striatum. Of the doses of DuP 996 tested (0.3 - 10 mg/kg s.c.) only 3.2 mg/kg produced a small (20%) increase in extracellular DA and 5-HT levels in rat striatum. High doses of DuP 996 (32 mg/kg) produced a more pronounced elevation (40-60%) in DA and 5-HT release, but extracellular levels of the metabolites DOPAC, HVA and 5-HIAA were increased to the same extent and all animals rapidly developed seizures. The neurochemical effects of high doses of DuP 996 could therefore be related to toxic events.

In view of the marked effects of DuP 996 on evoked DA release *in vitro*, we also studied its effects on extracellular DA levels during K^+ stimulation. To that end, 60 mM KCl was perfused for 60 min and the maximal increase in DA release was measured ("S1"). Three hours later the effects of a second KCl perfusion on DA release were assessed ("S2"), after pretreatment with 10 mg/kg s.c. DuP 996 or vehicle. Comparison of the "S2/S1" ratios for vehicle and DuP 996 treated animals, showed that the K^+ stimulated DA release was slightly, but not significantly, increased by DuP 996.

These *in vivo* results confirm that DuP 996 does not enhance the *basal* release of DA and 5-HT, but do not corroborate the pronounced effects of DuP 996 on *evoked* DA release *in vitro*.

774.4

PRE AND POSTSYNAPTIC SEROTONERGIC MARKERS IN A COMMUNITY ACQUIRED STUDY OF ALZHEIMER'S DISEASE.

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20 cases from a prospectively studied, community based sample of demented patients with neuropathologically confirmed Alzheimer's disease (AD) and 20 controls matched for sex, post-mortem delay and storage time were studied.

There was a significant loss of immunoreactive serotonergic dorsal and median raphe neurones in AD compared with controls, however this was not reflected in presynaptic serotonergic markers in the cortex. Thus there was no reduction in the concentration of 5-HT or 5-HIAA, as measured by HPLC, indeed the 5-HIAA/5-HT ratio was increased. Similarly, B_{max} for [^{3}H] paroxetine binding to the 5-HT reuptake site was only reduced in one of the two areas studied. AD cases treated with neuroleptics had reduced concentrations of 5-HT compared to untreated AD patients. Depressed AD patients had significantly lower paroxetine B_{max} compared to non-depressed AD cases. There was no significant difference in the number of 5-HT_{1A} receptors in AD compared with control, but 5-HT_{2A} receptor B_{max} was reduced, the K_D was unchanged in both cases. These data indicate that due to plasticity in the serotonergic system there is little evidence for a pre-synaptic deficit in AD and that reductions reported in previous studies may be due to drug treatment and the presence of non-cognitive behavioural changes such as depression.

An altered balance of postsynaptic receptors and a preservation of the presynaptic system may have implications for the treatment of AD.

774.5

THE DORSAL RAPHE AND DEPRESSION IN ALZHEIMER'S DISEASE.

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There is considerable evidence supporting a role of serotonergic systems as well as noradrenergic systems in the pathophysiology of major depression. We have previously demonstrated an association between major depression complicating Alzheimer's disease (AD) and disproportionate neuronal loss within the noradrenergic locus coeruleus (Soc Neurosci Abstr 1992;18:206). In order to investigate the possible association of serotonergic pathology and depression, we used an anti-phenylalanine hydroxylase antibody (PH8) (gift from RGH Cotton, Melbourne) to selectively label serotonergic neurons within the dorsal raphe (DR) nucleus of 5 patients with AD complicated by major depression (preliminary assessments based upon prospectively collected psychiatric evaluations) and from 8 age-matched patients without this complication. Sections (12 μm thick) of DR 1.2 mm and 1.68 mm caudal to the oculomotor nucleus were selected from serially sectioned formalin-fixed brainstems (transverse plane). Numbers of PH8-immunoreactive neuronal profiles greater than 12.5 μm in maximum diameter within defined boundaries of the DR were counted manually (400X). Mean profile counts tended to be nonsignificantly higher in depressed than in non-depressed patients at both anatomical levels evaluated: 177.8 ± 57.3 and 204.3 ± 61.4 in depressed patients versus 142.7 ± 51.6 and 179.9 ± 75.1 in patients without depression at rostral and caudal levels. Thus, there does not appear to be a relationship between loss of serotonergic DR neurons and depression in AD.

774.6

POLYAMINES IN HUMAN BRAIN: INFLUENCE OF REGIONAL DISTRIBUTION, AGING AND ALZHEIMER'S DISEASE.

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Despite considerable evidence implicating polyamines in brain development and CNS function, little information is available on these substances in normal or diseased human brain. We examined the influence of regional distribution, aging and Alzheimer's Disease (AD) on putrescine, spermidine and spermine levels in autopsied human brain. All three polyamines showed distinct and uneven distribution profiles amongst the 10 examined control brain areas. No statistically significant influence of aging (1 day to 103 years; n=57) on either putrescine or spermine levels in occipital cortex was observed. In contrast, spermidine levels increased markedly from birth, reaching maximal levels at approximately 40 years of age (+228% increase, $p<0.002$, one-way ANOVA, $r=0.71$ Pearson's correlation), which were maintained up to senescence. We also measured polyamine levels in cortical and subcortical areas of 12 patients with AD and carefully matched controls. Mean spermidine levels were markedly and significantly increased (70%) whereas putrescine levels were decreased (28%) in temporal cortex of AD patients compared with controls. No other statistically significant changes were observed. In view of the modulatory effects of polyamines on ion channels and glutamate receptor function, abnormal polyamine levels may contribute to excitotoxic processes occurring in brain of patients with AD. Supported by U.S. NIH NINDS grant NS26034 and the Hospital for Sick Children Foundation.

774.7

Vulnerability of pyramidal neurons immunoreactive for an N-acetylated dipeptide in Alzheimer's and Huntington's disease.

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An increasing body of evidence suggests a neurocommunicative role for N-acetyl-aspartyl-glutamate (NAAG), especially in putative glutamatergic pathways. Within glutamatergic pathways in cerebral cortex of primates, NAAG is preferentially localized to a subpopulation of pyramidal neurons. Subsets of pyramidal neurons associated with corticostriatal, corticothalamic and corticopontine projections have previously been described to exhibit NAAG-like immunoreactivity (NAAG-LI) in human brain (Passani et al., 1994). In the present study we investigated the impact of neurodegenerative disease on this type of NAAG-LI neuron. One characteristic feature of Huntington's disease (HD) and Alzheimer's disease (AD) is the selective degeneration of subsets of glutamatergic pyramidal neurons. In HD, losses are most prevalent in layer III and VI of frontal lobe and primary visual cortex and in layer IIIc of the temporal lobe. In AD, losses occur especially in layer III and V of the neocortex, and in the CA1 field and subiculum of the hippocampus. Our preliminary results suggest selective sparing of subpopulations of NAAG-LI pyramidal neurons in frontal cortex and primary visual cortex of HD post mortem brain. In both regions NAAG levels (determined by HPLC) and numbers of NAAG-LI pyramidal neurons (layer III, IVb, VI) are not significantly altered compared to control. Glutamate levels on the other hand are decreased up to 70%, suggesting either loss of glutamatergic neurons or metabolic alterations. In AD, NAAG levels are unaltered in hippocampus and in frontal, primary visual and temporal cortex. These findings suggest different degrees of vulnerablility for glutamatergic pyramidal neurons depending on the presence or absence of NAAG.

774.8

NON-PLAQUE DYSTROPHIC DENDRITES IN ALZHEIMER HIPPOCAMPUS: A NEW PATHOLOGICAL STRUCTURE REVEALED BY GLUTAMATE RECEPTOR IMMUNOCYTOCHEMISTRY.

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Alzheimer's disease (AD) is a progressive dementia characterized by a pronounced neurodegeneration in entorhinal cortex, hippocampal CA1 region and subiculum. Excitatory amino acid (EAA) receptor-mediated excitotoxicity is thought to play a role in the neurodegeneration in AD CA1. The present study investigated immunocytochemical localization of EAA receptor subunits in the hippocampus from 10 AD and 10 controls, matched for age, sex and post-mortem interval. Immunocytochemistry with antibodies specific for GluR1, GluR2(4) (AMPA), GluR5/6/7 (kainate) and NR1 (NMDA) receptor subunits demonstrated virtually all projection neurons in all subfields contained subunits from each receptor class. In CA1 GluR1, GluR2 and NR1 labeling was in pyramidal cell somata and dendrites. GluR1, GluR2(4) and NR1 immunolabeling revealed a novel pathological structure in all AD, but none of the control samples.The lesions were juxtacellular clusters of granular immunoreactivity in the neuropil of the pyramidal cell layer. Counterstaining for amyloid indicated lesions were not senile plaques. Neurofibrillary tangles and granulovacuolar degeneration were also not immunolabeled. Ultrastructural analysis revealed the lesions to be dystrophic cellular processes containing dense vesicles and flocculent material with immunolabeling localized to plasma and vesicular membranes. Lesions were not associated with amyloid fibrils. One lesion also contained Hirano body filaments indicating that the dystrophic processes were dendritic in origin. Additional studies are needed to determine the pathogenesis of these novel, non-plaque dystrophic dendrites.

774.9

REGIONAL VARIATIONS IN THE NEURONAL GLUTAMATE TRANSPORTER PROTEIN IN ALZHEIMER DISEASE.

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The pathological features of Alzheimer disease (AD) are area-specific. Pharmacological and molecular biological studies have described several forms of the glutamate transporter protein. If the glutamate uptake system were altered in AD cases, this could enhance excitotoxicity, particularly if the variant transporter protein was inefficient at removing glutamate from the synaptic cleft. Several damaged areas (showing neuronal loss and neurofibrillary tangles) were compared with relatively spared areas to determine how glutamate transporter protein sites vary between control and pathologically confirmed AD cases. We looked at the high-affinity uptake site using binding profiles for D-[^{3}H]aspartate and a range of transport inhibitors (unlabelled D-aspartate, L-glutamate, dihydrokainate, L-cysteate, *threo*-3-hydroxy-D,L-aspartate, L-α-aminoadipate, *cis*-1-aminocyclobutane-1,3-dicarboxylate and α-methyl-D,L-glutamate). Synaptosomal plasma membranes were prepared from human brain tissue obtained post mortem. Data from the homogenate binding studies were processed by the LIGAND program, and analyses of variance performed on logarithmic transforms of the affinity constants. Control and AD cases showed significant differences in transporter binding profile in motor ($P = 0.025$), occipital ($P < 0.001$), mid-temporal ($P = 0.035$), and cingulate regions ($P < 0.01$). The inferior temporal, parietal, hippocampal, and superior and inferior frontal areas did not differ significantly between controls and AD cases. Thus, different forms of the neuronal glutamate transporter may occur in AD cortex, in regions showing variable susceptibility to pathological change. Areas most resistant to damage tended to show greater variations in profile, and all showed a markedly enhanced affinity for glutamate in the AD cases.

774.10

NITRIC OXIDE SYNTHASE (NOS) IN POSTSYNAPTIC DENSITY FRACTIONS FROM CEREBRAL CORTEX OF RAT, NORMAL HUMAN AND ALZHEIMER'S DISEASE BRAINS.

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Nitric oxide synthase (NOS) catalyzes formation of the gaseous transmitter, nitric oxde (NO). Electron microscopic studies have revealed that neuronal NOS (nNOS) is an intrinsic component of the postsynaptic density (PSD), a disc-shaped specialization of the postsynaptic membrane. We now present biochemical evidence for nNOS expression within the PSD. We also show that nNOS levels in the PSD exhibit regional variation and may be elevated in Alzheimer's disease (AD).

We examined expression of nNOS in PSD fractions from adult rat and human brains. Western blot analyses, with a monoclonal antibody against nNOS, revealed higher levels of expression in PSD than in whole synaptic membrane fractions. nNOS levels in PSD samples from rat brain exhibited regional variation. These differences (Cerebellum > Olfactory Bulb > Hippocampus > Cerebral Cortex) reflect the reported distribution of nNOS in whole brain sections (Dinerman et al.,1994, PNAS *91*:4214).

Recent evidence suggests that Alzheimer's disease (AD) may be associated with biochemical changes in the PSD. We examined whether synaptic NOS might be affected in AD brains. In cortical PSD samples from two AD brains, PSD nNOS levels were elevated relative to controls. The pathophysiologic significance of elevated nNOS remains unclear.

Our findings indicate that nNOS is an intrinsic component of the isolated PSD. Regional variation in nNOS levels within PSD fractions may have consequences with respect to synaptic transmission in these areas.

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774.11

IMPAIRMENT OF THE PHOSPHOINOSITIDE SIGNAL TRANSDUCTION SYSTEM IN ALZHEIMER'S DISEASE BRAIN.

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Degeneration of cholinergic neurons with retention of postsynaptic muscarinic receptors in Alzheimer's disease (AD) has led to the widespread therapeutic use of cholinomimetics. The purpose of this study was to determine the functional capacity of muscarinic receptors linked to the phosphoinositide second messenger system using postmortem AD and matched control prefrontal cortex.

Activation of phosphoinositide hydrolysis was measured using membranes incubated with [^{3}H]phosphatidylinositol (PI). G-protein-coupled stimulation of phospholipase C was concentration-dependently activated by GTPγS and this response was 40% lower in AD than control membranes. Inclusion of carbachol to activate muscarinic receptors coupled with G-protein-mediated PI hydrolysis was impaired by 45% in AD membranes. Further analysis of the data indicated that the G-protein deficit in AD had a predominant influence on the lowered response to carbachol. Similar deficits in the responses to other cholinergic agonists were observed in AD membranes. A range of impairments were observed when non-cholinergic agonists were studied and when the responses to GTPγS and to cholinomimetics were measured in additional brain regions. These results demonstrate that there are severe deficits in the phosphoinositide signal transduction system in AD and that impaired G-protein function is an important contributory factor.

774.12

DIFFERENTIALLY ALTERED G-PROTEIN-MEDIATED SIGNALLING IN ALZHEIMER'S DISEASE: A STUDY OF STAGED AUTOPSY CASES

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(3) Neuropathologie. J. Gutenberg-Universität, 55131 Mainz, Germany

G-proteins are the interface between many receptors and effectors. In recent studies we showed that both stimulatory ß-adrenoceptors and inhibitory A1-adenosine receptors are unaltered in Alzheimer's disease (AD) (Lemmer et al., 1993). The ß-adrenoceptor-coupling to the stimulatory Gs-protein and the Gs-effector-coupling, was altered, whereas the inhibitory receptor/Gi/effector-coupling was intact (Schnecko et al., 1994). In the present study we determined hippocampal G-proteins by means of standardized Western-blotting in 12 AD-cases and 11 age- and post mortem delay-matched individuals with no clinical history of cognitive impairment. However, 6 meet criteria for stages II&III of AD-related neurofibrillary changes (Braak and Braak, 1991). Data are shown as as percent of control (stage 0) of standardized means.

Stage	Go	Gs	GsH	GsL	Gi
II/III	65.8	52.4*	37.9*	63.0	61.2
V (AD)	61.2	48.9**	42.8	50.8**	32.3**

(*=p<0.05, **=p<0.02 vs control; Kruskal-Wallis test)

There is no correlation between G-protein level and G-protein-mediated effector stimulation or inhibition.

Supported by the DFG (Oh48/1-2)

774.13

CALCINEURIN-LIKE PHOSPHATASE ACTIVITY IN CONTROL AND ALZHEIMER'S DISEASE STRIATE CORTEX. C.J. Ladner and J.M. Lee*.
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Neurofibrillary tangles found in the brains of individuals with Alzheimer's disease (AD) are composed of abnormally hyperphosphorylated forms of tau protein. The phosphorylation state of proteins is determined by the balance of kinase and phosphatase activities. Thus, the increase in tau phosphorylation may result from an increase in kinase activity and/or a decrease in phosphatase activity. The present study examined calcineurin-like phosphatase activity in homogenates of postmortem striate cortex from age matched controls (n=6) and AD cases (n=7). The homogenates were separated into particulate and cytosolic fractions. Phosphatase activity was assessed using the substrate p-nitrophenylphosphate (10 mM) and measured as a change in absorbance (λ=405 nm) due to the formation of p-nitrophenol. Measurements of phosphatase activity were determined in the presence or absence of calcineurin activators (1 mM $NiCl_2$ and 0.1 mM $CaCl_2$) and calcineurin inhibitors (200 μM trifluoperazine and 300 μg/ml cyclosporin A). In control particulate fraction, a significant increase in phosphatase activity was observed in the presence of nickel. The nickel-stimulated increase in phosphatase activity was antagonized by trifluoperazine and cyclosporin A. These biochemical properties of the particulate phosphatase activity are consistent with calcineurin. As a percentage of basal, nickel produced a 65% increase in phosphatase activity in control particulate fractions, but only a 24% increase in AD samples ($p<0.03$, student's t-test). Absolute values of basal activity did not significantly differ between control and AD samples. In the cytosolic fraction, no stimulation of phosphatase activity by divalent cations nor inhibition by TFP and CsA was observed. In addition, no differences between control and AD cytosolic samples were found. These findings suggest that particulate calcineurin-like activity may be reduced in striate cortex in AD. Current studies are examining the relationship between calcineurin activity with changes in neuropathology.

774.14

PROTEIN KINASE C LEVELS AND ACTIVITY IN CULTURED SKIN FIBROBLASTS FROM AFFECTED AND NON-AFFECTED MEMBERS OF THE SWEDISH FAMILY WITH THE AMYLOID PRECURSOR PROTEIN 670/671 MUTATION. M. Vestling*, A. Adem, L. Lannfelt and R. F. Cowburn.
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The familial Alzheimer's disease-causing amyloid precursor protein (APP) 670/671 double mutation has been shown to give a 3-fold increase of ß-amyloid production in cultured fibroblasts from affected individuals. Since APP processing can be regulated by protein kinase C (PKC) activation, we investigated whether the phenotype of the APP 670/671 mutation included a PKC deficit in primary skin fibroblasts.

PKC activities and levels were studied in the particulate and soluble fractions from 6 mutation bearing and 7 control cell lines. PKC activity in the soluble fraction, determined as the phosphorylation of endogenous histone substrate, showed an age-related decline ($r=-0.569$, $p<0.05$, Fisher's test) when data from all 13 cell lines was analysed together. No significant differences were seen in soluble PKC activity between mutation-bearing and control cell lines, the mean (± SEM) values being 251 ± 20.2 and 225 ± 21.4 pmols/min/mg protein, respectively. PKC activities in the particulate fractions were below the assay detection limit.

[^{3}H]Phorbol-12,13-dibutyrate binding studies revealed no significant differences in PKC levels between mutation-bearing and control cell lines when assays were performed using a ligand concentration of 10 nM. The mean (± SEM) PKC levels in the soluble fractions were 7.84 ± 0.50 and 6.56 ± 0.74 pmols/mg protein, respectively. The corresponding values for the particulate fractions were 2.39 ± 0.24 and 2.21 ± 0.15 pmols/mg protein.

It is concluded that the APP 670/671 mutation does not result in altered fibroblast PKC levels and activity. This is in contrast to the reported PKC deficits in fibroblasts from other sporadic and familial Alzheimer's disease cases.

774.15

AGING CAUSES SELECTIVE LOSS OF CALBINDIN-D_{28K} FROM THE CHOLINERGIC NEURONS OF THE HUMAN BASAL FOREBRAIN. C.-K. Wu[1*], M.-M. Mesulam[2] and C. Geula[1].
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Age-related changes in calbindin-D_{28K} immunoreactivity within the cholinergic neurons of the basal forebrain (BFCNs) were studied in the human brain and compared to changes in choline acetyltransferase(ChAT) and nerve growth factor receptor (NGFr) immunoreactivity. Calbindin-D_{28K}, ChAT and NGFr were visualized immunohistochemically in adjacent sections of the basal forebrain in 12 brains from normal individuals 20-91 years old. The BFCNs in the majority of individuals over 70 years (n=6 of 7) displayed a marked loss of calbindin-D_{28K} immunoreactivity when compared with individuals younger than 70 years of age (n=5). This loss was observed in all BFCN groups and reached a magnitude of 80%. ChAT and NGFr immunoreactivity, which are co-localized with calbindin-D_{28K} in these cholinergic neurons, displayed no similar age-related loss, indicating the selectivity of this observation. Loss of calbindin-D_{28K} would deprive cholinergic neurons of the capacity to buffer intracellular calcium levels and leave them vulnerable to processes which increase intracellular calcium and thus lead to neuronal death. The age-related loss of calbindin-D_{28K} from the BFCNs is a potential mechanism for the selective loss of these neurons in neurodegenerative diseases of the elderly, such as Alzheimer's disease.

774.16

INCREASED GLUCOCORTICOID (GR) AND MINERALOCORTICOID (MR) RECEPTOR mRNA EXPRESSION IN HIPPOCAMPAL SUBREGIONS FROM ALZHEIMER'S DISEASE PATIENTS. J.A. Davis* and R.M. Booze. Dept. of Pharmacology, Univ. of Kentucky, Lexington, KY 40536-0084.

Corticosteroids have been implicated in the pathogenesis of neurodegenerative disorders such as Alzheimer's disease. This study examined GR and MR mRNA expression in human post-mortem brain tissue from Alzheimer's disease (AD) patients and age-matched controls. Tissue was collected from 27 AD patients (age=79.5±1.5 yrs; 15 women, 12 men) and 10 control patients (age=75.3±2.8 yrs; 7 women, 3 men) from the ADRC at Sanders-Brown Center on Aging. Brodmann Area 9, hippocampus and cerebellar tissue was collected and immersion-fixed in 4% paraformaldehyde. Tissue blocks were cryoprotected and sectioned (40µm) at -17°C onto Vectabond coated slides. Stained and hybridized sections were counted (positive cells/100 µm^2) using the MCID M4 imaging system. Thioflavin-S stain confirmed the presence of amyloid plaques and neurofibrillary tangles in AD brains. Following non-isotopic *in situ* hybridization of GR and MR mRNA, significantly higher levels of MR mRNA expression were observed in Area 9 from AD patients relative to controls (18.65 cells/100µm^2 versus 7.50 cells/100µm^2). The CA3 region from AD patients exhibited a modest 44% elevation in MR mRNA expression compared to controls (32.72±2.51 versus 22.65±7.16 cells/100µm^2, respectively). Slightly elevated levels of GR mRNA expression was detected only in the CA1 region of AD brain (20.13±3.69 cells/100µm^2) compared to GR mRNA message expression in the CA1 region from control tissue (16.45±1.45 cell/100µm^2). This data suggests that altered expression of corticosteroid receptors could have a role in neurodegeneration observed with normal aging and AD. Progressive failure of calcium homeostasis initiated by glucocorticoid action is associated with cell loss in aging and AD. Our experiments indicate that individual variation in aging and AD is an important factor with regard to assessing mechanisms which underlie cell loss. (Supported by AG10836)

774.17

17α-ESTRADIOL EXERTS NEUROPROTECTIVE EFFECTS ON SK-N-SH CELLS. J.W. Simpkins, P.S. Green, J. Bishop and N. Tumer*, Center for the Neurobiology of Aging and the Departments of Pharmacodynamics and Pharmacology, GRECC, VA Medical Center, University of Florida, Gainesville, FL.

Estradiol (E2) has been shown to exert organizational, neurotrophic and neuroprotective effects in the central nervous system. The present study assessed the specificity of the neuroprotective effects of estradiol for the potent 17ß-isomer. SK-N-SH cells from a human neuroblastoma cell line which we have shown to be estrogen-responsive, were cultured at low or high plating density. Cells were then exposed to 17ß-E2 (0.2 or 2nM), 17α-E2 (0.2 or 2nM), cholesterol, testosterone, or dihydrotestosterone (all at 2nM), progesterone or corticosterone (0.2 to 200 nM). Cultures were insulted by serum deprivation, which caused a profound loss of cells. At 1 or 2 days of serum deprivation and steroid hormone replacement, the protection afforded cells by the steroid addition was assessed. Serum deprivation killed about 90% of cells cultured at both low and high plating density. Both 17α- and 17ß-E2 provided a dose-dependent protection of SK-N-SH cells at either plating density. By contrast, none of the other steroids tested protected cells from the insult of serum deprivation. The addition of progesterone (2nM) to cultures did not effect the cytoprotection observed with either isomer of estradiol at either dose. These results indicate that the neuroprotective effects of estrogens are not a general steroid effect and do not relate to the potency of estradiol isomers, as assessed by binding to cytosolic estrogen-receptors or responses of peripheral estrogen-responsive tissues. As such, the neuroprotective effects of estrogens may be mediated by a non-genomic mechanism. (Supported by NIH AG10485 and Apollo Genetics, Inc.)

774.18

EFFECTS OF U-101033E ON THE EXPRESSION OF AMYLOID PROTEIN PRECURSOR, APOLIPOPROTEIN E, GLIAL FIBRILLARY ACIDIC PROTEIN, AND B-AMYLOID EXPRESSION FOLLOWING A BILATERAL CAROTID OCCLUSION IN THE GERBIL. J.A.Oostveen*, D.B.Carter, E.Dunn, and E.D.Hall. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

Previously we have shown that a brief period of bilateral carotid occlusion (BCO)-induced forebrain ischemia in gerbils triggers a progressive expression of amyloid protein precursor (APP), apolipoprotein E (APO-E), glial fibrillary acidic protein (GFAP), and β-amyloid (β-AP) in the selectively vulnerable CA1 region of the hippocampus. The increase in immunoreactivity is secondary to the degeneration of the CA1 neurons. Oxygen radicals and lipid peroxidation (LP) have been demonstrated to play a role in post-ischemic neuronal damage, and recent literature suggests a possible link between early oxidative stress and subsequent APP expression. Therefore, the present investigation examined the effect of a novel brain penetrating pyrrolopyrimidine LP inhibitor (U-101033E) on APP, APO-E, GFAP, and β-AP expression. Gerbils were treated at 30 mg/kg p.o. 30 min prior to the BCO and 2 hrs post-ischemia, followed by daily dosing for the next three days. U-101033E provided a significant decrease in the expression of all markers ($p<0.05$), which correlated with a significant effect on preservation of the CA1 neuronal cell population ($p<0.05$) as determined by cresyl violet histochemistry. Lipid peroxidation inhibitors may provide attenuation of various response proteins to ischemic injury, probably through reduction of neuronal cell damage.

774.19

SUBSTANCE P POTENTIATES INTERLEUKIN-1β- AND LPS-INDUCED RELEASE OF IL-6 FROM HUMAN ASTROCYTOMAS. C.E. Nolan, K. Richter, M.A. Collins, and R.B. Nelson*. Department of Neuroscience, Pfizer Central Research, Groton, CT.

Astrocytes responding to optic nerve transection upregulate high-affinity substance P-binding sites (NK-1 receptors) within a month following the injury (PNAS 86:5193). The functional consequences of NK-1 receptor upregulation on reactive astrocytes are unknown. Because this receptor upregulation occurs in the context of an inflammatory response to brain injury, we sought to determine how substance P (SP) interacts with pro-inflammatory factors in modulating reactive astrocyte function. We first determined (as previously reported) that in U373 cells SP alone causes a moderate dose-dependent increase in both IL-6 release and intracellular calcium levels. Both effects are blocked by the non-peptide NK-1 antagonist CP-96345. The maximal SP-evoked IL-6 release is at least 10-fold *less* than the maximal release evoked with the pro-inflammatory agents IL-1β or lipopolysaccharide (LPS). However, we found that SP can interact synergistically with either IL-1β or LPS to increase IL-6 release. For example, 1 pM IL-1β and 100 nM SP added *separately* to U373 cells caused a total increased IL-6 release of 2250 pg/ml, but in combination produced an increase of 3350 pg/ml, a 43% synergism. A more dramatic synergism was found between 100 nM SP and and 10 μg/ml LPS where the increase in IL-6 release caused by the combination of both agents was 200% greater than the total release evoked for both agents added separately. This potentiation occurred over a limited dose range for both IL-1β and LPS and did not appear to be attributable simply to shifting the EC_{50}s for these agents. The present findings suggest that substance P may potentiate cytokine-evoked release of IL-6 from reactive astrocytes. Since astrocyte overexpression of IL-6 *in vivo* has been shown to produce neurodegeneration, NK-1 antagonists may have therapeutic application in neuropathologies involving chronic CNS inflammation, such as Alzheimer's disease, multiple sclerosis, and Parkinson's disease.

DEGENERATIVE DISEASE: PARKINSON'S—TRANSPLANTATION, PALLIDOTOMY AND IMAGING

775.1

TROPHIC SUPPLEMENTS OF MESENCEPHALIC CELLS IN HIBERNATION MEDIA ENHANCES SURVIVAL OF DOPAMINE NEURONS. P.M. Carvey*, L.R. Ptak and Z.D. Ling. Research Center for Brain Repair, Rush-Presbyterian-St. Lukes M.C., Chicago, IL 60612.

Mesencephalic neurons destined for transplantation into the striatum of patients with Parkinson's disease (PD), are generally held in hibernation (high K^+ salt solutions at 4°C) for several days prior to surgery. During this time, tissue viability can drop by as much as 10%/day. We evaluated the effects of supplementing the hibernation media with various trophic solutions including striatal extracts, human placental cord serum (HPCS), fetal calf serum (FCS), brain-derived neurotrophic factor (BDNF) and epidermal growth factor (EGF) to assess their effects on the survival of hibernated cells. E15 rostral mesencephalic tegmentum cells containing DA neurons were harvested, processed and immediately placed into hibernation media alone or with various trophic supplements. After 5 days the cells were washed, viability was assessed using trypan blue exclusion, and 100,000 viable cells were seeded onto established E15 striatal feeder cultures (100,000 cells/cm^2). After 72 hours, the cultures were processed for immunocytochemistry and the number of tyrosine hydroxylase immunoreactive (THir) neurons was assessed. All trophic supplements enhanced viability relative cells held in hibernation media only. This effect was not due to a stabilizing effect of protein on the media since bovine serum albumin did not alter viability. Striatal extracts, HPCS and FCS also enhanced the "survivability" of THir cells disproportionately. Thus, these trophic supplements not only increased survival in hibernation, but once plated out at equivalent seeding densities, a larger number of THir cells with more developed processes was observed. These data demonstrate that trophic supplements can enhance the viability as well as survival of DA neurons held in hibernation. Trophic supplements may reduce apoptosis initiated during storage thereby reducing the impact programmed cell death has on the survival of DA neurons following transplantation. (Supported by 1RO1NS33174).

775.2

ADDITION OF ADENOVIRAL VECTORS TO EMBRYONIC VENTRAL MESENCEPHALON CELL SUSPENSIONS USED FOR STRIATAL IMPLANTATION INTO 6-OHDA-DENERVATED RATS INDUCES DETRIMENTAL IMMUNE RESPONSES. E. Roy*, L. Grégoire, F. Tardif, P. J. Bédard and C. Gravel. Neurobiology Res Center, Fac. of Med., Laval Univ., Québec, Canada, G1J 1Z4.

As survival and differentiation of embryonic dopaminergic cells *in vitro* can be enhanced by exposure to neurotrophic factors, it may be possible to enhance survival and differentiation of dopaminergic donor cells *in vivo* in grafting trials by exposing them to an increased supply of such factors. In practice however, this is difficult to achieve and very invasive techniques such as intraparenchymal cannulation or coadministration of genetically-engineered cells are required. An alternative strategy would be to use gene transfer techniques to confer *in situ* increased expression of neurotrophic factors to donor and/or host cells. To test such a strategy, we have performed intrastriatal transplantation of embryonic ventral mesencephalon (VM) cell suspensions into the striatum of 6-OHDA-denervated rats using our standard protocols (Rioux *et al.*), with or without the addition of 3 x 10^7 pfus of E1-deleted adenoviral vectors encoding either E.coli LacZ (Ad5-CMV-LacZ) or human BDNF (Ad5-CMV-BDNF) in the inoculum. Striatal tissues were analysed 7 days after grafting. Addition of either adenoviral vectors to the donor suspension has detrimental effects on graft survival and differentiation, as illustrated by the reduced numbers of surviving TH+ cells and their restriction to a smaller area of the host striatum. Perivascular cuffs are observed and Mac-1 immunoreactivity is increased in the striatum of animals grafted with the suspensions supplemented with either vector, and GFAP+ cells are much less numerous in the graft in comparison to controls. We suggest that these effects result from the immune rejection of both grafted and host infected cells triggered by the presence of the adenoviral vectors in the tissues. Immune rejection of adenovirus-infected cells have been documented before by us and others in peripheral organs, but have not been reported yet in CNS tissues. We believe that the damage caused to the blood-brain barrier by the grafting procedure is instrumental in this rejection process, and we will suggest alternative strategies to prevent this rejection. [Supported by the MRC, the Parkinson Foundation of Canada and the Canadian Networks of Centres of Excellence.]

775.3

STRIATAL TRANSPLANTATION OF MICROENCAPSULATED BOVINE CHROMAFFIN CELLS REDUCES ROTATIONAL BEHAVIOUR IN THE RAT MODEL OF PARKINSONISM. P.W. Tsang, H.C. Kwan* and A.M. Sun. *Department of Physiology, University of Toronto, Toronto, ON, M5S 1A8, CANADA.*

Striatal transplantation of dopamine-producing tissue has been proposed as a possible treatment for Parkinson's disease. To avoid the ethical issues associated with the use of human fetal tissue, transplantation of chromaffin cells from the adrenal gland has been suggested; however, survivability of these transplants is quite low. In our study, we assess the efficacy of bovine chromaffin cell transplants immunoisolated within a permselective biocompatible polymer membrane vs. free cell transplants in the rat model of parkinsonism.

In vitro studies compared levels of high-potassium depolarization-evoked release of catecholamines from free chromaffin cells vs. cells microencapsulated within alginate/poly-lysine/alginate (APA) capsules. Results of a perifusion study showed comparable levels of release from both free and encapsulated cells; 1 x 10^4 encapsulated cells released approximately 4700 ng of noradrenaline, 5000 ng of adrenaline and 350 ng of dopamine. *In vivo* testing compared the effects of sham (empty capsule), free cells and encapsulated cells implanted into the striatum of male Wistar rats with unilateral 6-hydroxydopamine lesions of the substantia nigra. Lesions resulted in rotational behaviour upon administration of the dopamine agonist, apomorphine (APO). Weekly APO challenges revealed significant differences in the 3 experimental groups, with an average of 29.3 ± 3.6% reduction in the number of rotations in the sham group, 49.8 ± 4.6% reduction in the free cell group and 68.2 ± 4.4% reduction in the encapsulated cell group, in excess of 16 weeks post-transplantation (n_s = 5, n_f = 5, n_e = 6; one-way ANOVA, $p < 0.001$). The results demonstrate that the APA membrane allows the passage of secreted catecholamines out of the capsule. The use of immunoisolated cells may improve cell survival following transplantation, thus reducing rotational behaviour in our animal model.

This work was supported by the Medical Research Council of Canada.

775.4

CHARACTERIZATION OF HSV-1-EXPRESSED TYROSINE HYDROXYLASE IN A NON-DOPAMINERGIC CELL LINE. F. Serrano*, M.A. Bender†, W.F. Goins†, M.J. Zigmond, J.C. Glorioso† and T.G. Sherman. Department of Neuroscience and †Department of Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA 15260.

The delivery of foreign genes into CNS neurons may aid in the treatment of certain neurodegenerative diseases, such as Parkinson's disease. Herpes simplex virus (HSV-1) is one tool that demonstrates particular promise for this function. We have previously presented information on our design and construction of a non-replicating HSV-1 variant that utilizes the expression of a transcriptional activator (GAL4:VP16) as a means of prolonging targeted gene expression during viral latency in cells of the CNS (Soc. Neurosci. Abstract 79.19, 1994). What remains unknown, however, is information describing the full complement of expressed genes required for the phenotypic conversion of non-catecholaminergic cells into dopaminergic cells. To begin addressing this question, we are now investigating the kinetic variables of virally-derived tyrosine hydroxylase (TH) expressed in non-dopaminergic cells in culture. Vero cells, a stable line derived from monkey kidney cells, were infected with HSV-1 constructs expressing the TH gene, either alone or in combination with the GAL4:VP16 transactivator. TH-vector infected Vero cells produced detectable TH protein at 24 h post-infection, and this TH protein was shown to be enzymatically active in an *in vitro* assay for TH activity. Expressed TH was 4-fold increased in activity after 48 h of infection compared to 24 h. Co-infection of cells with the GAL4:VP16 virus produced no additional effect at 24 h; however, a 3-fold increase in TH activity was found after 48 hr compared to infection with the TH construct alone. Preliminary studies indicate that Vero cells expressing TH are capable of producing quantifiable levels of DOPA. (Research is supported by NINDS grant MH19608; F.S. by NIMH Training grant MH18273.)

775.5

LONG-TERM GENE EXPRESSION IN VIVO, DRIVEN BY A TYROSINE HYDROXYLASE PROMOTER IN A DEFECTIVE HERPES VIRUS AMPLICON VECTOR. M.Belloni, B.Conti, H.J.Federoff [1], B.K.Jin, T.H.Joh *. Cornell Univ.Med.Coll.at The Burke Med. Res. Inst., White plains, NY 10605., [1]Albert Einstein Coll. Med., Bronx, NY 10461.

Defective herpes simplex type 1 (HSV-1) viral vectors have been widely used for *in vivo* gene transfer in the central nervous system (CNS). Because the duration of gene expression directed by the viral promoters in amplicon vectors is short-lived, we constructed an amplicon vector in which gene expression was directed by the tyrosine hydroxlase (TH) promoter. A 9.0 kb fragment of 5' upstream DNA sequence of the rat TH gene, previously shown to direct tissue-specific expression in transgenic mice, was fused to LacZ gene in HSV amplicon (THlac). The viral IE4/5 promoter (HSVlac) was used as a control. Sprague Dawley rats received unilateral stereotaxic injections of virus stock into substantia nigra (SN). To determine retrogradely transported expression, virus stock was injected stereotaxically into striatum. After three weeks rats were sacrificed for X-gal histochemical and TH immunocytochemical analysis. In rats receiving nigral injections of THlac, expression of the LacZ gene was observed in neurons as well as glia. In rats receiving striatal injections, blue cells were also observed in SN ipsilateral to injection side. These blue cells were nigral dopaminergic neurons evidenced by double staining with TH immunocytochemistry. In contrast, neither nigral nor striatal injection of HSVlac resulted in prolonged gene expression. The present study suggests that a neuronal, but not a viral promoter, will direct longer-term expression in adult brain. Supported by NIH grant, MH24285.

775.6

CHARACTERIZATION OF FIBROBLAST CELLS GENETICALLY MODIFIED TO PRODUCE L-DOPA *IN VITRO* AND *IN VIVO*. C. Bencsics*, S. R. Wachtel, D. Young and U. J. Kang. Dept. of Neurology, University of Chicago, Chicago, IL 60637.

In previous studies, we have generated cells (FFK1THGC) which produce L-DOPA by doubly transducing primary fibroblasts with the cDNA's for tyrosine hydroxylase (TH) and GTP cyclohydrolase 1 (GTPCH1). These cells produce L-DOPA spontaneously since they produce tetrahydrobiopterin (BH4) which is the cofactor for TH.

To examine dopamine synthesis *in vitro*, FFK1THGC cells were cocultured with fibroblasts engineered to produce aromatic L-amino acid decarboxylase (FFK1AADC). Cocultured cells were incubated with media containing 0.01% of ascorbic acid for one hour. This media was analyzed for L-DOPA and dopamine (DA) by HPLC-ED. FFK1AADC and FFK1THGC cells cocultured with unmodified fibroblasts served as controls. FFK1THGC cells produced high levels of L-DOPA. When FFK1THGC cells were cocultured with FFK1AADC cells, there was a reduction in L-DOPA and dopamine was detected in the media.

These FFK1THGC cells were grafted into the striatum of rats depleted of DA by 6-OHDA. Immunostaining for TH in FFK1THGC cells was more robust compared to FFK1TH grafts, suggesting that BH4 may have another role in addition to acting as a necessary cofactor for its enzymatic activity. Further *in vivo* studies examining biochemical and behavioral effects of these cells are in progress.

775.7

THE EFFECTS OF A PEDICLED OMENTAL GRAFT TO THE BRAIN OF RATS WITH NIGROSTRIATAL LESIONS. N.S. Norton*, M. Brown, A.-A. Patil, A.J. Griess, B. Zumpano, and J.F. Rodriguez-Sierra. Dep't. of Cell Biology & Anat. and Div. of Neurosurg., Univ. of Neb. Med. Center, Omaha, NE 68198.

Parkinson's Disease is a debilitating illness characterized by degeneration of the dopaminergic neurons in the nigrostriatal system. The greater omentum possesses an abundant supply of dopamine and is capable of developing cortical vascular connections when transposed to the brain. We investigated the potential benefits of transplanting a pedicled graft of the greater omentum to the brain as a potential therapy in a Parkinson's animal model (PAM) since the omentum might increase the supply of dopamine to the corpus striatum and retard progression of the motor deficits. Sprague-Dawley male rats (60 days of age) were divided into four groups: 1) a control group, 2) a sham group, 3) a PAM Oil group, and 4) a PAM omental group. PAMs were produced by intracranial administration of 6-hydroxydopamine (6-OHDA). Apomorphine induced rotational studies were conducted weekly to monitor the progression of the motor deficit. Following two weeks of behavioral testing, the PAM Oil group received laparotomy followed by craniotomy over the area of the temporal lobe followed by closure of the wounds. The PAM omental animals received laparotomy and an omental flap with intact vascular pedicle, was brought to the interscapular area. Approximately one week after this surgical procedure, the proximal end of the pedicle was brought under the subcutaneous tissue and transposed to the surface of the cortical area at the level of the parietal-temporal lobe juncture. Apomorphine induced rotational studies were continued an additional three weeks. The results showed that the number of rotations was significantly less ($p<0.001$) in the PAM omental group when compared to the PAM Oil group. The results suggest that pedicled omental graft might be effective in ameliorating the progression of a PAM.

775.8

HOW FUNCTIONAL NEUROSURGICAL PROCEDURES WORK IN PARKINSON'S DISEASE: A THEORETICAL EXPLANATION. K. V. Baev*, K. A. Greene, A. G. Shetter, A. N. Lieberman. Barrow Neurological Institute, Phoenix, Arizona 85013.

In a previous publication (Baev K.V., Neurol. Res. 1994, v.17, 38-48) it was suggested that the skeletomotor basal ganglia-thalamocortical loop(s) can be considered as a functional system that models the behavior of the body and the environment during movement control. This predictory mechanism has to be tuned on the controlled object in order to function properly, *i.e.*, it has to be a learning system. The parameters of this system are tuned on the object behavior using an error signal that comes from the substantia nigra pars compacta. Minimization of the error signal is the final stage of learning process.

Therefore, Parkinson's disease (PD) has to be considered to result from degeneration of the error distribution system. Based on this theory, we make the conclusion that functional neurosurgical procedures such as pallidotomy or thalamotomy trick the controlling system in such a way that it does not recognize an error in its prediction anymore. Lesions placed in the pallidum or its projections to the thalamus make predictive signals less important so that real afferent flow from the controlled object prevails. After placement of a lesion in nonpallidal thalamic projections, the system primarily chooses predictive model afferent flow to determine the current state of the object. Therefore, these surgical procedures have to be considered symptomatic treatments: There is no real improvement of system function, only alleviation of the symptoms of PD. Chronic stimulation of basal ganglia circuitry through implanted electrodes has to be considered as adding noise to the system in addition to a functional block that it places at the stimulation site. Noise helps the system to slide down along an error surface to its global minimum when the model correctly describes the object behavior. Other methods of treatment of PD such as transplantation will be also discussed.

775.9

POSTEROVENTRAL PALIDOTOMY INDUCES MOTOR IMPROVEMENT AND DYSKINESIA IN MPTP-MONKEYS. M.R. Luquin*, J. Guillén, L. del Rio, J. Domínguez and C. Dávila. Department of Neurology, Clínica Universitaria de Navarra, Medical School, University of Navarra, Pamplona, Spain.

We have studied the effect of the lesion of the globus pallidus medialis (GPM) in 3 monkeys rendered parkinsonian by intravenous administration of MPTP. Monkeys were allowed to recover from the last MPTP injection up to 6 months and they did not receive any dopaminergic agents. Fine motor tasks were performed in all animals before MPTP administration and inmediately before surgery, when the degree of parkinsonism was considered stable. Unilateral posteroventral palidotomy was performed in 2 monkeys while in the other animal, the lesion was made in both GPM. The lesion of the GPM was made by injecting 1-2 µl of 50 nM kainic acid using a modified David Koff stereotaxic frame. Unilateral lesion of the GPM induced a contralateral amelioration of parkinsonism with a parallel improvement of fine motor tasks, but mild contralateral chorea was also observed, which persisted up to 3 months. Bilateral lesion of GPM induced severe generalized dyskinesia with improvement of parkinsonism. Post-mortem analysis showed the lesion was confined to the posterior part of the GPM, but a cell depletion was also observed in the dorsolateral part of the subthalamic nucleus homolateral to the lesion.

775.10

THE MECHANISM OF ACTION OF PALLIDOTOMY IN PARKINSON'S DISEASE (PD): CLINCAL AND PHARMACOLOGICAL RESPONSE. G. Linazasoro, J.A. Obeso, A. Gorospe, E. Ramos, R. Bakay, M. DeLong, J. Vitek*, Cliníca Quirón, San Sebastian (Spain) and Emory University Hospital, Atlanta, GA 30322

Six patients with PD were evaluated before and 3-6 months after microelectrode guided unilateral pallidotomy. Rigidity and akinesia were measured individually for each body segment, and arm and leg timed tapping tests were scored at baseline and after 24-72 hours without drugs. Pharmacological tests assessed the duration of the motor response (ON) to Sinemet (250/25 mg) and subcutaneous apomorphine (2,4,6,8 mg) and the severity and type of dyskinesias. Pallidotomy induced a marked reduction in akinesia and rigidity mainly contralateral to the lesion. The duration of the response to Sinemet was significantly prolonged and dyskinesias disappeared completely. Some body segments, i.e. shoulder, showed a permanent improvement after 24-72 hours without medication. Pallidotomy shifts the pharmacological response towards characteristics compatible with milder severity of parkinsonism. The response to dopaminergic drugs is not blocked by pallidotomy.

775.11

THE MECHANISM OF ACTION OF PALLIDOTOMY IN PARKINSON'S DISEASE (PD): PHYSIOLOGICAL AND IMAGING STUDIES. J.A. Obeso*, J.C. Rothwell, A. Ceballos-Bauman, N. Leenders, D. Brooks, P. Asselman, G. Linazasoro, J. Guridi, R. Bakay, J. Vitek, M. DeLong, Clínica Quirón, San Sebastian, Spain; MRC Motor and PET Units, London (UK), Paul Scherer Institute, Villingen (Switzerland), Emory University Hospital, Atlanta, GA

Six patients with PD were evaluated before and 3-6 months after microelectrode guided unilateral pallidotomy. Laboratory assessment included recording EEG premovement potential, magnetic cortical stimulation of the motor cortex, interonset latency (IOL) for performing sequential hand movements, simple and choice reaction time (RT), the long latency stretch reflex and the blink reflex. Positron Emission Tomography (PET) water scans (Oxygen-15) were taken while the subjects performed sequential hand movements. Following pallidotomy, all patients improved clinically but hand function remained unchanged in two. PET studies showed a significantly increased activation of the supplementary motor area (SMA), areas 6 and 4 and dorsolateral prefrontal cortex in 4 patients. The IOL for sequential hand movements was reduced by 44% and simple RT was decreased by 34%. There were no significant changes in the LLSR, blink reflex and motor cortex recovery curves and choice RT. Restoration of premotor and dorsolateral prefrontal cortex activity appears to be an important mechanism mediating the effect of pallidotomy in PD.

775.12

Characteristics Of 18F labelled ligands for Dopamine and dopamine Transport Function Using Positron Emission Tomography (PET). Bakay RAE*, Goodman MM, Boyer KL, Watts RW, Byrd L, Hoffman JM, Departments of Neurology and Neurosurgery, Emory University School of Medicine, Atlanta, GA 30322.

In order to develop and characterize Dopamine and Dopamine transport function in CNS transplantation by non-invasive techniques, a series of nonhuman primate studies were performed using PET. Fluorodopa is a standard for evaluating the dopaminergic system, but has a high signal-to-noise ratio. Dopamine transport complex is important in the regulation and synthesis of Dopamine, and is diminished in Parkinson's disease. Two distinct isotopes have been developed; fluoro-propyl-chloro-nortropane (FPCT) and fluoro-isopropyl-chloro-tropane (FIPCT). These compounds were synthesized to minimize nonspecific binding and achieve maximum specific activity. Nonhuman primate studies have been performed that characterize both blood and brain kinetic behavior of these isotopes. In combination with fluorodopa, it will be possible to completely characterize the uptake, binding, incorporation, release, and reuptake of Dopamine in vivo using PET. Data from normal, MPTP-lesioned, surgical controls and fetal mesencephalic grafted nonhuman primates will be presented. Supported by NS-24340 and R R-00165.

775.13

INCREASED DOPAMINE TURNOVER IN THE MPTP-INDUCED MODEL OF PARKINSONISM IN PRIMATES MEASURED BY PET WITH FLUORODOPA (FD). D.J. Doudet*, J.E. Holden, G. Chan, S. Morrison and T.J. Ruth. Div. Neurology, Dept. Medicine, Univ. British Columbia and UBC/Triumf PET, Vancouver, BC, Canada, V6T 2B5

The graphical analysis of PET data permits to estimate the striatal uptake rate constant (Ki) of FD. An extension of this graphical method made it possible to estimate the rate of loss of striatal signal (Kloss) during FD PET studies. Kloss is an index of the release, metabolism and outward diffusion of fluorodopamine and its metabolites. We examined the rate of loss of striatal signal in FD PET studies in normal and MPTP-treated rhesus monkeys to explore the hypothesis that dopamine (DA) metabolism is increased in parkinsonism.

Four unilaterally MPTP-treated rhesus monkeys and 4 age-matched controls were scanned in an ECAT 953B/31 tomograph for up to 4 hrs after injection of 5 mCi of FD. Metabolite analysis was performed using an alumina extraction method with anion/cation exchange columns. The extended graphical analysis was used to calcultate Kloss. Kloss was significantly ($p<0.05$) higher in the MPTP-treated striatum (0.00573 ± 0.002 min^{-1}) compared to the unlesioned striatum (0.00243 ± 0.00069 min^{-1}) and controls (0.00258 ± 0.00069 min^{-1}).

These results are in keeping with the hypothesis of compensatory increase in DA turnover in parkinsonism. Kloss as measured with FD PET may be used to explore DA presynaptic compensatory mechanisms.

775.14

[18F]6-FLUORO-L-M-TYROSINE: INITIAL STUDIES OF METABOLISM AND PHARMACOKINETICS. S. Jordan, J.L. Eberling*, H.F. VanBrocklin, J.P. O'Neil, K. Bankiewicz, W.J. Jagust. Center for Functional Imaging, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720: Somatix Therapy Corp. Alameda, CA 94501.

[18F]6-fluoro-L-m-tyrosine (FMT) has many favorable characteristics as a Positron Emission Tomography (PET) tracer of dopaminergic function. During dynamic PET imaging with FMT in 3 rhesus monkeys we measured plasma FMT and its F18-labeled metabolites 6-fluoro-3-hydroxyphenylacetic acid (FPAC) and 6-fluoro-3-hydroxyphenylethylamine (FMA). In a separate study, 4 animals were injected with FMT and euthanized 60 and 120 mins later to determine its metabolism in brain. In both studies, samples were separated by HPLC and fractions containing labeled FMT, FPAC or FMA were counted for F18 activity. Elution times were calculated for external standards by HPLC with electrochemical detection which enabled fraction identity. These blood and brain data were used to construct a kinetic model for FMT. The major FMT metabolite in plasma was FPAC, which together with FMA accounted for over 20, 30, 40 and 50% of total plasma activity at 10, 30, 60 and 120 mins after FMT injection, respectively. FPAC was also the predominant metabolite in 60 and 120 minute post-mortem brain samples, representing over 80% total activity in both caudate and putamen. Less metabolism occurred in regions containing few dopamine terminals, such as frontal cortex (over 30% total activity was FMT), and cerebellum (over 40% total activity was labeled FMT). A three rate constant fit to a three compartmental model of FMT uptake, using the metabolite-corrected blood input function with the dynamic PET brain uptake data, revealed higher k3 values in both caudate (0.06±0.01) and putamen (0.05±0.003) than frontal cortex (0.002±0.001). Taken together these data support the use of this tracer in studying dopaminergic function in parkinsonian primates following neurografting and gene therapy.

775.15

PET [18F]6-fluoro-L-m-tyrosine Imaging of MPTP-lesioned Primates. W.J. Jagust*, J.L. Eberling, S. Jordan, H.F. VanBrocklin, J.P. O'Neil, M. Emborg, D. Rosenberg, K.S. Bankiewicz Center for Functional Imaging, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720; Somatix Therapy Corporation, Alameda, CA 94501.

PET studies using the dopamine tracer, [18F]6-fluoro-L-dopa (F-dopa) have been used extensively to study Parkinson's disease. Interpretation of these studies has been limited because of the presence of methylated metabolites that readily enter the brain. Here we report the results of PET studies using the tracer [18F]6-fluoro-L-m-tyrosine (FMT) in 3 monkeys before and at weekly intervals after unilateral intracarotid artery (ICA) and i.v. MPTP administration. Animals were injected with approximately 10 mCi of FMT and were imaged for approximately 2 hours. Prior to MPTP administration, all animals showed preferential accumulation of the tracer bilaterally in the striatum and midbrain, but little accumulation in other brain regions. Following unilateral ICA MPTP administration (3 mg MPTP-HCl), we observed decreased signal in the striatum on the lesioned side and increased signal in the nigra/VTA complex. After i.v. MPTP administration (0.3 mg/kg) we observed a further reduction in signal in the striatum on the previously lesioned side, as well as a reduction on the contralateral side. Data were analyzed using a 3 compartment model with 3 rate constants to describe the time course of radioactivity in the striatum. Prior to MPTP administration the mean value for k3, describing FMT decarboxylation, was 0.06 (sd = 0.01) for the putamen. Following unilateral ICA MPTP administration, k3 for the putamen was 0.003 (sd = 0.001) for the lesioned side, and 0.04 (sd = 0.01) for the contralateral side. After i.v. MPTP administration there was a further decrease in k3 for the previously lesioned putamen (mean k3 = 0, sd = 0.0001), as well as a decrease on the contralateral side (mean k3 = 0.02, sd = 0.01). These values corresponded to the extent of progressive clinical deterioration. FMT produces robust estimates of dopaminergic function which are effective for tracking MPTP lesions and should be helpful in detecting grafts of genetically modified cells in this model.

775.16

ELEVATION OF TYROSINE HYDROXYLASE (TH) mRNA EXPRESSION AND FLUORO-META TYROSINE (FMT) SIGNAL MEASURED BY POSITRON EMISSION TOMOGRAPHY (PET) IN THE SUBSTANTIA NIGRA (S.N.) AFTER MPTP ADMINISTRATION IN MONKEYS. D. Nagy*, J. L. Eberling#, W. J. Jagust#, S. Jordan#, M.E.Emborg, W.W McLaughlin and K.S.Bankiewicz. Somatix Therapy Corporation, Alameda, CA 94501, #Lawrence Berkeley Laboratory, UC Berkeley, CA 94720.

Administration of 0.4 mg/kg of MPTP into one internal carotid artery (ICA) produces hemiparkinsonism (HPD) in monkeys (ms). In this study we examined TH mRNA expression in normal and 4 HPD+ ms, while 3 other ms. underwent PET scanning after FMT administration before and at weekly intervals after ICA and i.v. MPTP administration. The ms received an intracarotid infusion of 3 mg of MPTP-HCL supplemented with 0.3mg/kg i.v. to induce general parkinsonian signs in addition to HPD. These animals were able to sustain themselves without any L-DOPA administration. The in-situ hybridization (ISH) technique was applied for the detection of the TH mRNA content of the midbrain DA cells using ^{35}S labeled complementary RNA probes. Adjacent sections were stained for H&E and examined for TH-immunoreactivity (TH-IR). In the SN on the side ipsilateral to MPTP infusion very few TH-IR cells were present and contralateral SN appeared to be partially lesioned. TH mRNA expression was found higher in the SN on the partially lesioned side of the treated animals, than in the controls. In all monkeys examined with PET there was an increase of signal in SN at 7 days after ICA MPTP on treated side and at 7 days on the contralateral side after i.v. MPTP injection. High levels of the TH-mRNA expression in the partially lesioned SN cells of the MPTP-treated animals and increase of FMT signal at 7 days after MPTP administration suggests up-regulation of the remaining DA cells. These findings are consistent with increased HVA/DA ratio in striatum after MPTP administration, and indicate compensatory mechanism of the remaining and/or degenerating DA cells in SN.

775.17

[123I]ß-CIT SPECT MEASURES ARE HIGHLY REPRODUCIBLE IN PARKINSON'S PATIENTS AND HEALTHY CONTROLS.

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The monoamine transporter ligand, [123I]ß-CIT and SPECT demonstrate significant reduction of striatal uptake in Parkinson's disease (PD) patients compared with age and gender-matched controls. In order to assess the utility of SPECT for serial evaluation of disease progression in PD we performed test/retest reproducibility studies in 7 healthy subjects (HS) and 7 PD patients. **Methods:** Subjects underwent SPECT imaging at 18, 21, and 24 h post intravenous injection of 10 mCi (370 mBq) of [123I]ß-CIT. SPECT scans were repeated 7-21 days later. The ratio of specific striatal:nondisplaceable uptake (designated V_3") was obtained. Using this measure, the test/retest variability and reliability were calculated using standard statistical methods. **Results**: Both PD and HS groups demonstrated low test/retest variability for V_3" with mean measures of 12.4±4.7% and 6.8±6.8% for PD and HS groups, respectively. There were no significant differences in variability in HS and PD patient groups (p=0.28). Similarly, calculation of reliability (where 1=total reliability) showed no differences in PD and HS controls with measures of 0.99 and 0.96, respectively. These data suggest [123I]ß-CIT SPECT provides reproducible measures of DA transporters in PD patients and supports the feasibility of using the tracer for within-subject evaluation of disease progression.

775.18

A NOVEL SPECT IMAGING AGENT DETECTS TOTAL LOSS OF THE DOPAMINE TRANSPORTER IN PARKINSONIAN MONKEYS.

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The dopamine transporter, localized on striatal neurons is an effective marker for Parkinson's disease. The present study highlights a novel SPECT (single photon emission computerized tomography) ligand, [123I]altropane™, that displays favorable characteristics for imaging the dopamine transporter. In human post-mortem striatum, [125I]altropane binds with high affinity (K_D: 4 nM) to the dopamine transporter and dopamine:serotonin transporter selectivity. In monkey striatum, SPECT imaging reveals selective accumulation of the ligand in striatum within 30 min and a striatum:cerebellum ratio > 10. Reversible binding is rapidly reduced by the dopamine transport inhibitor WIN 35,428. In MPTP-induced Parkinsonism, [125I]altropane binding is undetectable. These studies highlight [123I]altropane as a SPECT imaging agent for the dopamine transporter and its potential for diagnosing and monitoring dopamine neurons in Parkinson's disease and other disorders in which dopamine transporter/neurons densities are modified. NS30556, Boston Life Sci. Inc., DA06303, DA09642, MH14275, RR00168.

NEUROTOXICITY: HEAVY METALS

776.1

ALTERATIONS OF LONG-TERM POTENTIATION (LTP) AND PAIRED-PULSE POTENTIATION (PPP) IN VIVO RAT HIPPOCAMPAL DENTATE FOLLOWING DEVELOPMENTAL LEAD EXPOSURE

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Dept. of Biology, Univ. Sci. Tech. China, P.R.China

Neonatal Wistar rats were exposed to lead from parturiution to weaning via the milk of dams drinking 0.2% lead acetate solution. The alterations in LTP and PPP of dentate gyrus in adult rats following developmental lead exposure were studied in vivo. Excitatory postsynaptic potentials (EPSPs) and population spikes (PSs) were recorded in the dentate in response to stimulation applied to the perforat path. The results showed that the LTP was induced in Pb-exposed rats with an average PS potentiation of 170±40.9%(n=11), which was significantly smaller than the increase in PS potentiations in control rats (325± 104%,n=13) (p<0.05) after tetanizing stimulation. The mean EPSPs amplitudes increased to 140.8±21.4% (n=11) for Pb-exposed rats and 174.4±7.0% (n=13) for the controls after tetanizing stimulation. The lead-induced alteration in LTP of PS potentiations was greater than that of EPSP potentiations. Paired-pulse potentiation (PPP) across a range of interpulse intervals is also depressed in Pb-exposed rats. Following pairs stimulation of perforant fiber at 250µA and an interpulse interval of 60ms, PPP for Pb-exposed rats was 157.0±42.0%(n=11) and for control rats was 213.0 ±54.0%(n= 13). Compared with controls, the mean PPP decreased 26.1% (p< 0.05). The results of this investigation demonstrated that the lead exposure in neonatal rats caused impairments in LTP and PPP of dentate gyrus. Supported by NNSFC (DYR)

776.2

ROLE OF APOPTOSIS IN DELAYED NEURONAL DEATH FOLLOWING A BRIEF EXPOSURE OF PRIMARY CULTURES OF CEREBELLAR GRANULE NEURONS TO ZINC.

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In primary cultures of rat cerebellar granule neurons, a brief, 15 min exposure to zinc (300 µM and higher) resulted in delayed neuronal death. Cell death was assayed by measuring mitochondrial viability with the MTT assay and cell membrane integrity with the trypan blue exclusion assay. These assays did not reveal any toxicity immediately after exposing the cultures to zinc; they became positive 4 to 24 h after transient zinc treatment. The toxicity of zinc depended on maturity of neuronal cultures - it was not apparent prior to day 5, and it reached a plateau around 9-10 days *in vitro*. The latter two days in vitro were selected for further experiments. A co-treatment with zinc and calcium channel blockers (nimodipine and amlodipine) reduced the toxicity of zinc. The latter results suggest that zinc may have triggered calcium-dependent mechanisms of delayed neuronal death. One of such mechanisms is apoptotic (programmed) cell death. We assayed the cultures for the presence of DNA fragmentation (a marker of apoptosis) using the *in situ* TUNEL technique, and found an increase in the number of apoptosis-positive cells after zinc treatment. However, actinomycin D, an inhibitor of macromolecular synthesis, failed to prevent zinc toxicity. The results suggest that a calcium channel blocker-sensitive increase in endonuclease activity without a concomitant induction of protein synthesis may be operative in zinc neurotoxicity.

776.3

NEUROTRANSMITTER RELEASE IN PC 12 CELLS EXPOSED TO LEAD.

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In children, exposure to low levels of lead (Pb) results in learning deficiencies. One hypothesis to explain this observation is that Pb impairs learning by disrupting mechanisms that control the release of neurotransmitters. The relationship between Pb and neurotransmitter release was examined by studying norepinephrine (NOR) release in PC 12 cells by using a method that takes advantage of the reuptake of [3H]NOR into vesicles. A 60 min incubation with 20 uM $(CH_3COO)_2Pb$, but not the chloride salts of Cd, Zn or Mn resulted in the release of NOR. The minimum concentration of Pb needed to provoke NOR release was 20 uM. However, if the cells were stimulated with inhibitors of microsomal Ca-ATPase or with activators of protein kinase C (PKC) the minimum concentration of Pb was reduced to 10 uM and 5 um, respectively. Neurotransmitter release was also accompanied with the release of two proteins found in secretory granules, secretogranin II and chromogranin B. A 200 uM concentration of Cd or Ni effectively blocked the release of NOR in PC 12 cells stimulated with an inhibitor of Ca-ATPase but was relatively ineffective in cells stimulated with a PKC activator. We conclude that PC 12 cells exposed to Pb release neurotransmitter. Furthermore, there appears to be at least two pathways that will heighten a cells's sensitivity to Pb, one that involves microsomal Ca-ATPase and another that uses PKC.

776.4

LOW LEVEL LEAD EXPOSURE DURING DEVELOPMENT: DOSE-DEPENDENT RUNWAY MAZE IMPAIRMENTS IN LABORATORY MICE TESTED IN EARLY ADULTHOOD.

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We investigated the effects of incremental doses of low-level Pb acetate or sodium acetate exposure during development on runway maze performance in Binghamton heterogeneous Stock (HET) mice. Mice were either untreated or received 0, 5, 10 or 25 mg/kg Pb acetate via intragastric intubation on postnatal days 6,9,12,15 & 18. No overt effects of Pb on physical development, including growth, were detected in any groups. When tested at 40-50 days of age, Pb-exposed mice exhibited a dose-dependent increase in the proportion of runway errors - with effects emerging at levels as low as 10 mg/kg. These data support growing evidence that even very low levels of Pb exposure during development may impair cognitive performance later in life.

776.5

LACK OF TOXICITY OF HUMAN NEUROMELANIN TO THE RAT BRAIN. P. L. McGeer[1], Y. Aimi[1] and T. Kawamata[2]*. 1: Kinsmen Lab. of Neurol. Res., University of British Columbia, Vancouver, B. C. Canada, V6T 1Z3. 2: Department of Neuropathology, Hyogo Institute for Aging Brain and Cognitive Disorders, Himeji, Japan, 670.

The prominence of neuromelanin in humans, and its accumulation extracellularly in Parkinson disease and other extrapyramidal disorders involving the substantia nigra, has led to speculation that neuromelanin might itself be neurotoxic. Iron is known to accumulate in the substantia nigra of Parkinsonian patients, and it has been shown that neuromelanin binds iron. Therefore, it has been even more specifically suggested that iron is neurotoxic when complexed with melanin. The proposed mechanism is oxidative stress, leading to neuronal death from toxic oxygen species. In order to test this hypothesis, as well as to learn the phagocytotic capability of microglia for neuromelanin, we extracted neuromelanin from the human substantia nigra of a normal and parkinsonian case. The extracted melanin was stereotaxically injected into the corpus striatum and substantia nigra of rats. Control injections were done with saline or brain extracts from non-pigmented brain regions. Rats were sacrificed by perfusion with aldehyde fixative at various periods after injection (3 days - 8 months). Brain sections were immunohistochemically stained with anti-tyrosine hydroxylase (TH) to reveal dopaminergic cells and processes, and OX 42 (anti C3b) to reveal microglia. Injected melanin remained even at 8 months following injection. Although some TH positive neurons were destroyed by trauma in the needle tract area in both control and melanin injected brains, TH positive neurons appeared to be completely unaffected by melanin at all time periods. There was no difference in results between normal and Parkinsonian melanin. Some melanin could be seen within a few OX42 positive microglia at all time periods, but most microglia seemed to ignore the melanin granules. The present results show that human neuromelanin is not toxic to rat substantia nigra or caudate-putamen neurons and it remains for long time periods in brain.

776.6

MANGANESE DECREASES GLUTAMATE UPTAKE IN CULTURED ASTROCYTES. A.S. Hazell* and M.D. Norenberg. Departments of Pathology and Biochemistry & Molecular Biology, University of Miami School of Medicine and VA Medical Center, Miami, FL 33101.

Manganese has recently been shown to accumulate in the basal ganglia in patients with chronic hepatic encephalopathy (HE). Ammonia and astrocytes have been strongly implicated in the pathogenesis of HE, and since we have recently shown that ammonia decreases glutamate uptake in cultured astrocytes, we examined whether manganese also affected glutamate uptake. Cultured astrocytes from newborn rats were treated with manganese (II) chloride (1-50 μM) in the absence or presence of ammonium chloride (5 mM), and glutamate uptake was determined using the non-metabolized glutamate analogue [^{3}H]-D-aspartate. Exposure times varied between 1 hr and 2 days. Treatment with manganese (50 μM) for 2 days resulted in a 11% decrease in transport of radiolabel into cells compared with controls (control 11.13 ± 2.37 vs. Mn^{++} 9.91 ± 2.66), while treatment with ammonia alone resulted in an 18% decreased uptake of [^{3}H]-D-aspartate after 48 hrs. In the presence of both manganese and ammonia, a further lowering of D-aspartate uptake was observed (34%). Preliminary data also suggested greater sensitivity of striatal cultures to the effects of manganese on glutamate transport. These results suggest that manganese alone has a detrimental action on glutamate transport into astrocytes which is additive to the ammonia effect, and may contribute to the pathogenesis of hepatic encephalopathy. (Supported by NIH grants DK38153, NS30291, VA and GRECC)

776.7

INHIBITION OF NMDA RECEPTORS BY LEAD IS DEPENDENT ON THE RECEPTOR SUBUNIT COMPOSITION. I.A Omelchenko, C.S. Nelson, J.L. Marino and C.N. Allen* Cntr. for Res. on Occup. & Environ. Toxicol., Ore Hlth Sci Univ, Portland, OR 97201.

Pb^{2+} is a potent inhibitor of NMDA receptors and its action is dependent on the age of neuron. We tested the hypothesis that the vulnerability of NMDA receptors to Pb^{2+} blockade is dependent on the receptor subunit composition which changes during development. Concentration-response curves for NMDA and glutamate were generated in the presence and absence of Pb^{2+} using the two electrode voltage-clamp technique and NMDA receptors consisting of $\zeta 1\varepsilon 1$, $\zeta 1\varepsilon 2$ and $\zeta 1\varepsilon 1\varepsilon 2$ subunit combinations expressed in *Xenopus laevis* oocytes. For all three subunit compositions glutamate was a more potent agonist than NMDA. In oocytes expressing $\zeta 1\varepsilon 1$, $\zeta 1\varepsilon 2$ and $\zeta 1\varepsilon 1\varepsilon 2$ subunits Pb^{2+} inhibited glutamate-activated currents with IC_{50}s of 0.87 ± 0.25 μM; 1.21 ± 0.22 μM; 6.1 ± 0.55 μM and NMDA-activated currents with IC_{50}s of 1.37 ± 0.47 μM; 1.11 ± 0.33 μM; 6.64 ± 1.5 μM (mean $\pm$ S.E.). Pb^{2+} reduced the maximal current amplitude but did not significantly alter the EC_{50}s for glutamate or NMDA consistent with a noncompetitive block. The IC_{50}s for Pb^{2+} blockade of NMDA- or glutamate-activated currents were significantly larger for $\zeta 1\varepsilon 1\varepsilon 2$ subunits when compared to $\zeta 1\varepsilon 1$ or $\zeta 1\varepsilon 2$ combinations. These data suggest that brain regions with $\zeta 1\varepsilon 1$ or $\zeta 1\varepsilon 2$ NMDA receptors subunits would be more vulnerable to Pb^{2+} toxicity than those with $\zeta 1\varepsilon 1\varepsilon 2$ NMDA-receptors. (Supported by NS19611)

776.8

ALTERATIONS IN N-METHYL-D-ASPARTATE (NMDA) RECEPTOR SUBUNIT mRNA AFTER LEAD TREATMENT IN NEONATAL RATS. M.A. Wilson*, J. Bressler, M.V. Johnston and G.W. Goldstein. Neuroscience Laboratory, Kennedy Krieger Research Institute and Dept. of Neurology, Johns Hopkins University, Baltimore, MD 21205

One of the primary sites of action of the environmental neurotoxin lead is at the NMDA receptor (Alkondon et al, '90); lead selectively inhibits NMDA currents, acting as a non-competitive antagonist of both NMDA and glycine (Guilarte & Miceli, '92, Uteshev et al., '93). We have examined the effects of neonatal lead exposure on NMDA receptor subunit expression in rat pups. Rat dams were administered 0.2% lead acetate in their drinking water, beginning on the day when their litters were delivered. Lead-treated and untreated control litters were culled to 8 pups at birth. Rat pups were decapitated on postnatal day 15, and *in situ* hybridization with oligonucleotide probes for NR1, NR2A and NR2B was used to evaluate NMDA receptor subunit expression in the hippocampus. Expression of NR2A mRNA was significantly reduced in the granule cell layer of the dentate hilus ($p<0.01$) and in the pyramidal layer of the CA1 region of the hippocampus ($p<0.05$). No significant changes in NR1 or NR2B expression were observed. The selective effect of lead on dentate granule cells is of special interest because these neurons have a mechanism for accumulation of the putative co-transmitter zinc that may serve to concentrate lead in this part of the hippocampus. Because NR2A expression normally increases in the forebrain between postnatal day 7 and 15, the reduced expression observed in lead treated rats may represent a developmental delay in maturation of excitatory amino acid receptors. (Support: The Robert Leet and Clara Guthrie Patterson Trust, NIEHS Grant -02380.)

776.9

BRAIN LIPID PEROXIDATION AND POLYDIPSIA DURING IRON OVERLOAD IN RAT. Carol A. Gunnett, James L. Hargrove, and Diane K. Hartle* Depts. of Pharmacology/Toxicology and Foods and Nutrition, The University of Georgia, Athens, GA 30602.

Brain membranes are composed of a high percentage of polyunsaturated fatty acids relative to most other tissues. The brain is therefore highly peroxidizable and at risk for oxidative free radical damage if local oxidative stress overcomes the brain's relatively moderate level of antioxidant defenses. The integrity of the blood-brain-barrier is essential to protect the brain continuously from oxidative stressors in the blood. Free iron catalyzes lipid peroxidation via Fenton reactions in vivo. Iron-catalyzed free radical formation initiates lipid peroxidation. The purpose of this study was to produce a short term oxidative stress on brain and peripheral organs using ferrous chloride (20 mg iron/kg, i.p.) and determine the extent of 1) lipid peroxidation and 2) glutathione levels in brain, liver, kidney and heart. Summary of results: Iron overload treatment caused rapid and profound polydipsia. The polydipsia was initiated within minutes of injection. Iron overload produces significant brain, liver, kidney and heart lipid peroxidation. Early time points had higher levels of lipid peroxidation relative to the 2 hr time period, presumably following rapid sequestration of free iron. Glutathione levels were not depleted in brain. GAagrES GAES # GE633 to JLH, AFPE Fellowship to CAG and Ga AHA.

776.10

EFFECTS OF *IN VIVO* CHRONIC LEAD EXPOSURE ON CA^{2+} CURRENTS IN BASAL FOREBRAIN NEURONS FROM ADULT RATS. C.A. Grover*, M.C. Jasek, W.H. Griffith, & G.D. Frye, Dept. of Medical Pharmacology & Toxicology, HSC, Texas A&M University, College Station, TX 77843-1114.

It is well known that *in vitro* acute lead treatment inhibits neuronal calcium currents. We studied the effects of chronic *in vivo* lead exposure on LVA and HVA calcium currents in basal forebrain neurons. Adult male Sprague Dawley rats were given 500 ppm lead acetate (Group Lead) or pair-fed sodium acetate (Group Control) in the drinking water for 65-75 days prior to *in vitro* electrophysiological experiments. Whole-cell patch-clamp techniques were performed on acutely isolated neurons from the basal forebrain. Current-densities were determined for all cells by normalizing Ca^{2+} currents (pA) for capacitance (pF), and current-voltage relationships were determined. From a holding potential of -80 mV Ca^{2+} currents were generated using a 2 sec prepulse to either -100 or -50 mV followed by voltage steps between -90 to +30 mV. There was no significant difference in maximum peak current-density between cells from Control ($X = 60.9 \pm 5.11$ pA/pF, n=20) and Lead ($X = 55.0 \pm 5.15$ pA/pF, n=27) treated animals. In a subset of cells rundown of maximum steady-state current-densities was significantly greater for Control ($X = 56.60 \pm 6.65$ %, n=10) than Lead rats ($X = 22.68 \pm 10.26$ %, n=10). These findings may suggest that chronic exposure to lead, like acute intracellular application of lead (Sun et al., *Soc. Neurosci. Abstr.*, 20:1719; 1994), causes a reduced rate of calcium current rundown. Supported by ES05639 (CAG), AA06322 (GDF) and AG07805 (WHG).

776.11

METHYLMERCURY (MeHg)-INDUCED INHIBITION OF WHOLE CELL POTASSIUM (K^+)-CURRENT IN RAT CEREBELLAR GRANULE CELLS. JE Sirois* and WD Atchison. Dept. Pharmacol./Toxicol., Inst. of Environ. Toxicology and Neuroscience Program, Michigan State University, East Lansing, MI 48824

MeHg is a neurotoxic metal which interacts with Na^+ and Ca^{2+} channels in a potent and apparently irreversible manner. Cerebellar granule cells are particularly sensitive to the *in vivo* effects of MeHg and provide an excellent model for studying MeHg effects on ion channels *in vitro*. The whole-cell patch clamp method was used to examine both peak and steady-state K^+-currents following culture of neonatal rat cerebellar granule cells for 7-12 days *in vitro* and to measure the effect of MeHg on these currents. From a holding potential (H_p) of -70mV, 250ms duration pulses to +30mV were given every 5 seconds. MeHg (2, 20 or 40 μM) inhibited K^+-currents in a concentration-dependent manner following exposure. Block was slow in onset and incomplete. At 2 μM MeHg was generally without effect, even following longer exposures. At 20 and 40 μM MeHg typically blocked both peak and end currents in an incomplete manner and also decreased the leakage current elicited by hyperpolarizing pulses from the H_p. The extent of block was similar following either strong (+120mV) or relatively weak (+10mV) depolarizations, demonstrating that the effect is not voltage-dependent. Initial experiments designed to examine the effect of MeHg on K^+-current activation and inactivation showed that MeHg did not alter the voltage at which the current activates, nor did it affect the rate at which the current inactivated. Thus, cerebellar granule cell K^+-channels do not appear to be a sensitive target of MeHg following *in vitro* exposure. Supported by NIH grant ES03299.

776.12

PATHWAYS MEDIATING Ca^{2+} ENTRY INDUCED BY METHYLMERCURY IN CEREBELLAR GRANULE CELLS. M. S. Marty* and W. D. Atchison. Dept. of Pharm. & Tox., Michigan State University, East Lansing, MI 48824.

Methyl mercury (MeHg) is a known neurotoxicant, hypothesized to cause cerebellar granule cell degeneration by altering Ca^{2+} homeostasis; however, the mechanism of this effect is poorly understood. Prior work demonstrated a MeHg-induced biphasic rise in intraneuronal Ca^{2+} ($[Ca^{2+}]_i$) with a large component of this rise due to influx of extracellular Ca^{2+}. This report examines potential route(s) of Ca^{2+} entry into granule cell cultures (7-10 DIV) after *in vitro* MeHg exposure. In contrast to results in NG108-15 cells, the dihydropyridine nifedipine did not significantly delay the time-to-onset of MeHg-induced elevations in $[Ca^{2+}]_i$, indicating that the routes of Ca^{2+}/MeHg entry in the two cell types may differ. Because excitatory amino acid (EAA) receptor-operated channels play a role in excitotoxicity, we hypothesized that granule cells are more susceptible to the effects of MeHg due to the presence of EAA receptor-operated channels. Thus, granule cells were exposed to 0.5 μM MeHg with and without various EAA inhibitors and changes in $[Ca^{2+}]_i$ were measured using fura-2. 6,7-Dinitroquinoxaline-2,3(1H,4H)-dione (DNQX, 100 μM), a non-N-methyl-D-aspartate (NMDA) receptor antagonist, inhibited 83% of the kainate-induced elevations in $[Ca^{2+}]_i$ but caused no delay in MeHg-induced $[Ca^{2+}]_i$ elevations. Similarly, 10 μM MK-801 and 100 μM 2-amino-5-phosphonovaleric acid (AP-5), antagonists capable of inhibiting 87% and 82% of the NMDA-induced elevations in $[Ca^{2+}]_i$, respectively, were ineffective at delaying MeHg-induced $[Ca^{2+}]_i$ elevations. These results show that EAA receptor-operated channels do not appear to be the route of MeHg/Ca^{2+} entry in granule cells *in vitro*. Supported by NIH grant ES03299.

776.13

MICROMOLAR CONCENTRATIONS OF METHYLMERCURY BLOCK VOLTAGE-ACTIVATED CALCIUM-, SODIUM- AND POTASSIUM CHANNELS OF RAT DRG-NEURONS IRREVERSIBLY. R. Leonhardt, H.L. Haas and D. Büsselberg★. Physiology II, H.-Heine-University, 40225 Düsseldorf, Germany.

Methylmercury (MeHg) readily accumulates in the nervous system and is known to cause a wide variety of neurotoxic effects, ranging from abnormal reflexes to loss of coordination and learning deficits.

Using the whole-cell patch-clamp technique, we examined voltage-activated ion currents of rat dorsal root ganglion (DRG) neurons. Cells were obtained from 2-4 day old rat pups and cultured for up to four days. Voltage-activated calcium-, sodium- and potassium channel currents were separated by selective blocking agents and specific depolarizing voltage steps. Calcium channel currents were carried by barium (10 mM). Subtypes of the three ion currents have not been distinguished. MeHg was applied by a bath perfusion system, it was added to the extracellular solution just before the beginning of each experiment. All currents were leak corrected by a P/4 protocol. Dose-response relationships were calculated by fitting the data to the Langmuir-equation.

All three types of voltage activated ion currents were reduced by MeHg in a concentration-dependent manner. Voltage-activated calcium- (IC_{50} ~2.6 μM) and potassium channel currents (IC_{50} ~2.2 μM) were ~5 times more sensitive to MeHg than voltage-activated sodium currents (IC_{50} ~12.3 μM). The calculated Hill-coefficients were ~1 for the blocking of calcium- and potassium channels and ~1.7 for the blocking of sodium channels. The reduction of calcium- and sodium channel currents appeared to be use-dependent. Independently of the external solution used, in some cases the application of higher concentrations of MeHg (≥5 μM) resulted in a biphasic change of the resting membrane current. None of the above described effects was reversible. These results indicate, that the blocking of voltage-activated ion channels may contribute to the neurotoxicity of MeHg.

776.14

DIFFERENTIAL EFFECTS OF Hg^{2+} AND METHYLMERCURY (MeHg) ON EXCITATORY AND INHIBITORY TRANSMISSION IN HIPPOCAMPAL SLICE. Y. Yuan* and W.D. Atchison. Dept. of Pharmacol./Toxicol. and Neurosci. Prgm. Michigan State University, E. Lansing, MI 48824.

Effects of Hg^{2+} and MeHg on resting membrane potentials, excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) were compared in CA1 neurons of rat hippocampal slices using intracellular microelectrode recording techniques. Acute bath application of Hg^{2+} and MeHg (20 or 100 µM) caused biphasic effects on resting membrane potentials, *i.e.* they initially hyperpolarized and then depolarized CA1 neuronal membranes. However, Hg^{2+} appeared less potent in depolarizing hippocampal neuronal membranes than is MeHg. Similarly, Hg^{2+} is less potent at blocking EPSPs than is MeHg. In contrast, both Hg^{2+} and MeHg blocked IPSPs more rapidly than they blocked EPSPs at similar concentrations. However, single-electrode voltage clamp recordings of IPSCs showed that Hg^{2+} and MeHg had different effects on the time-courses and the current-voltage relationships. MeHg suppressed amplitudes of inward and outward currents at all holding potentials and shifted the I/V curve to more positive potentials, *i.e.* changing the reversal potential from about -75 mV (control) to more positive values. Hg^{2+} also suppressed both inward and outward currents, but it initially caused an increased outward current prior to suppressing it. Moreover, Hg^{2+} appeared to shift the I/V curve to more negative potentials and its overall effects on IPSC were much slower than those of MeHg. Thus, these results suggest that the mechanisms involved in these actions of Hg^{2+} and MeHg may be different. Supported by NIH grant ES03299.

776.15

STUDIES ON THE MECHANISM OF METHYLMERCURY (MeHg) TRANSPORT INTO THE BRAIN. R. Park, S. Yee and B. H. Choi*. Neuropathology, Univ. of California, Irvine, CA 92717.

Previous studies in our laboratory as well as those of others have demonstrated that transport of MeHg into brains of mature rats is enhanced by co-administration of L-cysteine. However, no significant enhancement of brain Hg uptake was noted following injection of 0.1 mM cysteine 20 minutes prior to 0.05 mM MMC injection in C57BL/6J mice. Whereas injection of ^{35}S-cysteine-MMC conjugate significantly enhanced brain cysteine uptake, separate injections of 20 mg/kg body weight of MMC and 30 µCi of ^{35}S-cysteine at 20 min. interval showed no significant enhancement of brain cysteine uptake as compared to controls. To further examine the mechanism of MeHg transport into the brain, varying doses (1, 2, 4 and 6 mM) of methylmercuric chloride (MMC), MMC-cysteine, MMC-glutathione (GSH) and MMC-β-mercaptoethanol (βME) were injected intra-peritoneally into C57BL/6J mice, and brain Hg uptake determined 3 hours thereafter. Dose-dependent increase of Hg uptake was noted in all groups. However, highly significant enhancement of Hg uptake took place in groups injected with MMC-conjugates (MMC-βME>MMC-cysteine>MMC-GSH>MMC). The kinetics of brain Hg uptake between MMC and MMC-βME groups also differed considerably. These data indicate that size and polarity of MMC-conjugates greatly influence MMC transport into the brain, and that MeHg transport into mature brains appears to take place through diverse and complex uptake systems. (Supported in part by NIEHS grant ES 02928).

776.16

CHRONIC EXPOSURE TO INORGANIC LEAD MODIFIES PKC ACTIVITY IN THE DEVELOPING RAT HIPPOCAMPUS. M. Madden & V. Miletic*. Dept. Comp. Biosci. & Environmental Toxicology Center, Univ. Wisconsin, Madison, WI 53706

The aims of this study were to characterize the subcellular distribution of protein kinase C (PKC) activity in the developing rat hippocampus, and to examine whether chronic lead exposure modifies this development. Dams were exposed to either 0 or 1000 ppm lead acetate in their drinking water and mated. Offspring were exposed *in utero*, via lactation, and directly in the drinking water after weaning. The offspring were sacrificed at postnatal days 1, 8, 15, and 29. Blood lead levels were determined at these postnatal periods and correlated to the amount of lead given. Total PKC activity was determined in the cytosolic and crude P-2 membrane fractions from each animal's hippocampus by an *in vitro* radioactive phosphorylation assay, and was expressed as picomoles of phosphate incorporated per mg of protein per min. In control rats, total PKC activity in the cytosolic fraction increased three-fold between P1 (1110 ± 283) and P8 (3296 ± 721) ($p<0.0005$), remained at these levels at P15, and then decreased somewhat at day 29 ($p<0.0025$). PKC activity in the membrane fraction of these normally developing rats also increased between P1 and P8 ($p<0.0005$), but then decreased at P15 ($p<0.0005$), and did not further change at P29. In lead-treated rats, PKC activity in the membrane fraction was significantly lower than in control rats at all measured postnatal periods ($p<0.01$ at P1, $p<0.0005$ at P8, P15, P29). In these same rats, cytosolic PKC activity was lower than in control animals at P8 and P29 ($p<0.0005$), but not at P1 and P15. These results indicate a significant modification of total PKC activity, especially in synaptosomal membrane fractions of lead-treated rats. This suggests that the modification might contribute to lead's neurotoxic action in the developing rat hippocampus. (Supported by NIH NS21278).

776.17

PRENATAL AND POSTNATAL CHRONIC EXPOSURE TO INORGANIC LEAD ATTENUATES LTP IN THE ADULT RAT HIPPOCAMPUS *IN VIVO*. A.Z. Elliott* & V. Miletic. Dept. Comp. Biosc. & Environ. Tox. Cntr., Univ. Wisconsin, Madison, WI 53706

We examined whether prenatal and postnatal chronic exposure to inorganic lead modifies the expression of long-term potentiation (LTP) in electrophysiological recordings in the adult rat hippocampus *in vivo*. Dams were given 0, 100, 500, or 1000ppm lead acetate in their drinking water beginning 2 weeks before mating. Offspring were exposed to lead via the milk and, after weaning, in the drinking water. At about 13 weeks of age, a male and a female from each litter were anesthetized with urethane (1.2-1.5 g/kg), and prepared for conventional recording of hippocampal field potentials. A bipolar stimulating electrode was lowered into the CA3 region, and the glass recording electrode (0.5-2MΩ) was positioned in the CA1 region until a maximum response to the CA3 stimulus was observed. LTP was induced by tetanic stimulation (one 400ms train of five 50ms pulses at 50Hz), and recordings of field potentials were repeated at 0.5, 1, 2, 3, and 4 hours post-tetanus. In control and 100ppm lead-treated animals (blood levels of 11.5μg/dL) an increase in the population spike (PS) amplitude of 250-300% over baseline levels was observed to persist through 4 hours. Animals given 1000ppm lead (blood levels of 31.5μg/dL) showed little LTP (PS increase <50%). Those exposed to 500ppm (blood levels of 24.6μg/dL) exhibited LTP (200% over baseline) at 1 hour, but then the PS declined to baseline levels. These data indicate that chronic exposure to lead resulting in blood levels as low as 25μg/dL prevents the full expression of LTP in the CA1 region of the rat hippocampus *in vivo*. The data further suggest that the failure to fully express LTP may contribute to lead's neurotoxic action in the developing rat hippocampus. (Supported by NIH NS21278).

776.18

CHRONIC LOW LEVEL LEAD EXPOSURE ALTERS CALCIUM CURRENT IN PHEOCHROMOCYTOMA (PC12) CELLS. C. C. Hegg* & V. Miletic. Dept. Comp. Biosci. & Environmental Toxicology Center, Univ. Wisconsin, Madison, WI 53706.

Lead is a known neurotoxin with varied and poorly understood mechanism(s) of action. We employed the whole-cell patch-clamp technique to examine the chronic effects of low level lead exposure on high-threshold voltage-dependent calcium currents in rat pheochromocytoma (PC12) cells. PC12 cells were exposed to 0, 10 or 50 μM lead-containing growth media for up to 8 weeks. At least four days prior to recording cells were plated in dishes in lead-containing media with nerve growth factor to induce differentiation. As previously reported in acute studies, chronic low level lead exposure caused a dose-dependent *decrease* in calcium current throughout a 4 week exposure. In contrast, after 5 - 8 weeks of lead exposure, both the peak and sustained components of the calcium current were *increased*. When the chronically exposed cells were further challenged with an additional 5 minute exposure to 1 μM lead a dose-dependent decrease in calcium current and an alteration in the activation and inactivation kinetics were observed. The effects of lead were only partially reversible with washing. These data suggest complex interactions of lead with calcium channels in PC12 cells. (Supported by NIH NS21278 & T32 ES07015).

776.19

EFFECTS OF *IN UTERO* METHYLMERCURY EXPOSURE ON HIGH AND LOW LUMINANCE VISUAL CONTRAST SENSITIVITY IN ADULT MONKEYS. S.G. Gilbert*, D.C. Rice, M.L. Mihali, C. Munkers, K.S. Grant-Webster and T.M. Burbacher. Dept. Environmental Health, Univ. of Washington, Seattle, WA 98195.

Both animal and human studies have shown that the visual system is sensitive to the effects of methylmercury (MeHg). The present study assessed visual function in adult monkeys (*Macaca fascicularis*) following *in utero* MeHg exposure. Maternal exposure of either 0, 50, 70, or 90 μg/kg/day of MeHg resulted in blood mercury levels at birth from 1.04 to 2.45 ppm for treated infants. There were 10, 9, 2 and 2 offspring produced, respectively. At approximately 9 to 11 years of age the monkeys were tested on an operant visual psychophysics task designed to assess spatial visual function at low and high luminance. A forced choice tracking procedure was utilized in which the monkeys faced two oscilloscope screens of equal average luminance and were required to press the button corresponding to the screen displaying a vertical sine wave grating. The following spatial frequencies were assessed: .5, 2, 4, 10, 20, and 30 cycles per degree of visual angle for high luminance and 1, 2, and 4 c/d for low luminance. Preliminary data from 12 of the monkeys at high luminance showed that some MeHg monkeys exhibited a decreased contrast sensitivity at high spatial frequency. There appear to be no treatment related effects at low luminance. That some treated individuals appear to exhibit greater visual impairment than others is consistent with previous studies of monkeys exposed to MeHg. These preliminary results, indicate that *in utero* MeHg exposure may adversely affect the spatial visual function of adult monkeys.

776.20

EFFECTS OF INTRASTRIATALLY ADMINISTERED LEAD ON EXTRACELLULAR FLUID (ECF) AMINO ACID CONCENTRATIONS IN THE STRIATUM: A MICRODIALYSIS STUDY. P. Chaiyakul*, J. Waraska, I. N. Acworth, and T. J. Maher. Division of Pharmaceutical Sciences, Massachusetts College of Pharmacy, Boston, MA 02115 and ESA Inc., Chelmsford, MA 01824.

Previous studies in our laboratory have shown that pretreatment with lead (Pb^{2+}) decreased the basal striatal ECF levels of aspartate (by 56%), glycine (by 18%) and taurine (by 23%), while increasing the K^+-evoked release of inhibitory amino acids (glycine and taurine) and decreasing excitatory amino acids (glutamate and aspartate). To further this line of investigation, we measured the ECF amino acid levels of rats intrastriatally perfused with artificial CSF (aCSF) containing concentrations of Pb^{2+} (0.1, 0.3, and 1.0 mM) using *in vivo* microdialysis. Urethane-anesthetized rats were stereotaxically-implanted with a 3 mm loop-design probe in the striatum and baseline samples collected every 20 min. for 2 hours. A Pb^{2+} containing aCSF (Mg^{2+} lowered accordingly to preserve isotonicity) was then perfused through the microdialysis probe using a liquid switch for 120 minutes. Compared to controls, aCSF containing Pb^{2+} (0.1 and 0.3 mM) significantly increased the ECF levels of all amino acids studied, but not in a dose-dependent manner. The highest concentration of Pb^{2+} (1.0 mM), where Mg^{2+} is decreased to 0 mM, significantly decreased the ECF levels of all the studied amino acids compared to controls. These data demonstrate a biphasic dose-response relationship between intrastriatal Pb^{2+} and ECF amino acid levels, suggesting that the observed changes in ECF amino acids may have partly resulted from alterations in aCSF Mg^{2+} concentrations. Additional studies on the role of Mg^{2+} and other divalent cations in the release and uptake of amino acid neurotransmitters are warranted.

776.21

REDUCED ChAT mRNA EXPRESSION IN SEPTUM OF POSTNATAL RATS FOLLOWING PERINATAL LOW-LEVEL LEAD EXPOSURE. X.Sun, X.Tian*, and J.B.Suszkiw. Dept. of Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH 45267-0576.

We investigated the effect of Pb-exposure on ChAT mRNA expression in postnatal rats. Rat pups were maternally Pb-exposed by giving 0.2% lead acetate in drinking water to dam. Septal tissue was homogenized in RNAzol ™ B (1:20, w/v). Total RNA from both control and Pb-exposed animals was extracted, quantified at OD_{260}, loaded at 20, 10, 5, 2.5, and 1.25 μg onto nylon membrane in Dot-blot manifold apparatus, and hybridized with γ ^{32}P ATP-radiolabeled 43bp ChAT antisense oligonucleotide probe or 21bp rat 18S rRNA probe, respectively. The blots were scanned and quantified with the aid of a phosphor imager SF (Molecular Dynamics). The ChAT mRNA signal was normalized to the corresponding signal obtained with a probe specific for the 18S ribosomal RNA. Relative to control levels, the ChAT mRNA in septa of PN21 and PN28 animals with Pb-exposure were reduced by 42% and 44%, respectively. These results indicate that the previously reported reduction of ChAT activity following Pb-exposure reflects the reduction of the ChAT mRNA levels. *This work was supported by NIEHS grant ES06365.*

776.22

EFFECTS OF LEAD ON CATECHOLAMINE BIOSYNTHESIS IN PC12 CELLS. A.M. Ndifor,R,R.Reams& K.Soliman*. College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307.

We have previously reported that lead does not modify dopamine β hydroxylase but induces a slight enhancement in tyrosine hydroxylase activity in PC12 cells. We have further studied the effects of lead acetate on the synthesis storage and release of dopamine (DA) and norepinephrine (NE). Cells were exposed to 0.3-25μg/dl Pb^{2+} for up to 3days and the levels of DA, NE and 5HT in the cells determined by HPLC. The release of these amines and their corresponding metabolites was also observed. Pb^{2+} significantly inhibited the release of NE but not DA. It also reduced metabolic deactivation of DA to 3,4 dihydroxyphenyl acetic acid and homovanillic acid. 5HT conversion to 5-hydroxyindolacetic acid was also inhibited. DA and NE storage was only marginally reduced by lead. The inhibitory effects of lead were most prominent after 24hrs exposure and were not significant at 72hrs, suggesting the presence of an equilibrating process. These results confirm Pb^{2+} as a potent inhibitor of oxidative metabolic enzyme activity, which could result in increased accumulation of the parent compounds with potential potentiation of their neuroactivities.
Supported by A
STDR #US50/ATU398940-02

777

SYMPOSIUM. OPIOIDERGIC MODULATION OF LONG-TERM POTENTIATION IN THE HIPPOCAMPUS: INSIGHTS INTO PEPTIDERGIC REGULATION OF SYNAPTIC PLASTICITY. C.R. Bramham, Univ. Bergen (Chairperson); C. Chavkin, Univ. Washington; J.L. Martinez, Jr., UC Berkeley; R. Nicoll, UCSF.

Opioid peptides are co-localized with the excitatory neurotransmitter glutamate in several hippocampal pathways. As is the case for most neuropeptides, a function for opioids has been difficult to define. Rapid advances have now established endogenous opioid peptides as major regulators of long-term potentiation (LTP) in the hippocampus. Similar to neuropeptides co-stored with classical transmitters in the peripheral nervous system, maximal release of opioid peptides occurs during high-frequency stimulation. Evidence indicating that activation of opioid, but not NMDA, receptors is required to induce LTP of opioid-containing pathways, including the lateral perforant path input to granule cells and CA3 pyramidal cells and the mossy fiber input to CA3 pyramidal cells, will be presented. Conflicting evidence that naloxone does not block mossy fiber LTP induction will also be discussed (Nicoll). The latest findings reveal an unexpected and intriguing complexity to opioid effects: dynorphins can act presynaptically to dampen excitatory transmission and inhibit LTP induction. Furthermore, dynorphins may be released from granule cell dendrites to act as retrograde neurotransmitters. The objective of the symposium is to synthesize what we know about the mechanisms and unique properties of opioid receptor-dependent LTP on the one hand (Bramham and Martinez), and inhibitory control of LTP by dynorphins on the other (Chavkin and Nicoll).

778

SYMPOSIUM. NEURAL CONTROL OF BREATHING. J.E. Remmers, Univ. of Calgary (Chairperson); D.W. Richter, Univ. of Gottingen; J.L. Feldman, UCLA; N.I. Syed, Univ. of Calgary.

The overall objective of this symposium will be to provide fundamental and comprehensive insights into both the developmental and comparative aspects of the cellular basis of respiratory rhythm generation. The first presentation will consider cellular, membrane, and metabolic factors that modulate the respiratory rhythm in adult mammals. The second presentation will review cellular and molecular mechanisms underlying respiratory rhythmogenesis in a slice preparation. The third presentation will focus on comparative aspects of respiratory chemoreception and rhythmogenesis in amphibia. The last presentation will deal with a novel approach of reconstruction of a central respiratory pattern generator and its modulation by neurotransmitters.

The approach of this session will encompass both neonates and adults in animals ranging from mammals, lower vertebrates to invertebrates. The session will be a unique opportunity to learn about the cellular basis of rhythm generation in phylogenetically and ontogenetically different species from a single ion channel to the whole animal behavior.

COGNITION XII

779.1

P300 LATENCY AT CORTICAL SITES IN VISUAL AND AUDITORY ODDBALL PARADIGMS. Ivy M. Dise Dunn*, Psychology Dept. & Beckman Institute, Univ. of Illinois at Urbana-Champaign, Champaign, IL 61820.

P300 latency was mapped during a topographic analysis of normal subjects. The objective was to determine if there is a consistent sequence in which P300 reaches peak amplitude across the cortex.

The P300 component was elicited with auditory and visual oddball paradigms in ten normal subjects. Peak latencies were ranked to show the sequence in which 18 scalp sites reached maximum amplitude. While individual variation occurred among subjects, several consistencies were seen.

The typical PZ-maximum distribution was present along the midline. In addition, a large P300-like peak occurred between 240ms - 590ms at sites away from the midline. This peak reached maximum amplitude at some sites simultaneously, but was delayed as much as 125ms at other locations. In the auditory condition, the central/parietal regions had the earliest latencies, and the latest latencies were frontal. P300 arose anteriorally in the visual condition, and terminated posteriorally. These results suggest that P300 reaches peak amplitude across the cortex in an orderly sequence. This sequence may provide insight into the cognitive processing associated with P300.

779.2

MODULATION OF FRONTAL- AND PARIETAL-LOBE ACTIVITY BY FOCUSED ATTENTION. PART I: METHOD AND PHASIC (SUB-SECOND) EFFECTS. *A.S. Gevins* & M.E. Smith. EEG Systems Laboratory & SAM Technology, One Rincon Center, San Francisco, CA, 94105.*

The strategic allocation of attention required to perform difficult cognitive tasks is reflected in corresponding phasic and tonic changes in human brain electrical activity. At present, high resolution electrophysiological methods are the only means of resolving both sub- and multi-second attentional effects in a single experiment. This may be done by analyzing the data both as discrete averaged Evoked Potential (EP) peaks, and as continuous unaveraged EEGs. An experiment suitable for both viewpoints was designed around demanding spatial and verbal tasks that required continuous maintenance and updating of representations in working memory (WM). Improved spatial resolution was obtained by recording EEGs with 115 electrodes, registering the data with anatomical models derived from each (N=8) subject's MRI, and correcting for volume conduction blurring through the skull and scalp, in effect estimating the potential distribution just above the cortical surface. For frequent, nontarget stimuli, a positive EP peak at 305ms was larger in the spatial WM condition relative to the other conditions, followed by a peak at 450ms that was larger in both spatial and verbal WM conditions relative to control conditions. The voltage maxima for the P305 and the P450 occurred over dorsalateral frontal cortex near the principal sulcus of the right- and left- hemispheres respectively. These brief (~100-200ms) events occurred in parallel with a positive slow wave, maximal over the superior parietal lobe and the supramarginal gyrus, with a slight right-hemisphere predominance. It began ~200ms after stimulus onset, returned to baseline by ~600ms post-stimulus in control conditions, and was sustained for ~1sec longer in the WM conditions than in control conditions. These results suggest that the phasic allocation of attention during WM tasks involves the momentary functional coordination of regions of frontal and parietal cortex. The relationship of these phenomena to multi-second tonic attentional processes is discussed in Part II (Smith & Gevins, this mtg.). This research was supported by NIMH, AFOSR, & ONR.

779.3

MODULATION OF FRONTAL- AND PARIETAL-LOBE ACTIVITY BY FOCUSED ATTENTION. PART II: TONIC (MULTI-SECOND) EFFECTS AND CONCLUSIONS. *M.E. Smith* & A.S. Gevins. EEG Systems Laboratory & SAM Technology, One Rincon Center, San Francisco, CA, 94105.*

When difficult tasks impose a sustained cognitive load, phasic brain electrical events are accompanied by tonic alterations in the ongoing EEG. To characterize these multi-second changes, the dataset described in Part I (Gevins & Smith, this mtg.) was spectral analyzed as 4 second epochs corresponding to single trials. Two distinct differences were reliably observed between working memory (WM) and control task conditions. First, over a restricted prefrontal region, power in the 4-7Hz (theta) band was increased in the WM tasks relative to the control tasks ($p < .02$ at peak signal intensity measured at a midline location over the superior frontal gyrus). Anatomically-registered dipole models of brain electrical currents localized the source of this signal in or near the anterior cingulate gyrus. Second, over widespread areas of cortex, but most pronounced over parietal regions, peak power in a 9-13Hz (alpha) band also differed between between WM and control tasks, with lower power in the WM conditions ($p < .001$ at a midline location over the posterior margin of the superior parietal lobule). Taken together with the Part I results, these findings suggest that a distributed network of attentional processes, with distinct timecourses in distinct neuronal populations, are required to perform the demanding WM tasks. In the frontal lobes, in conjunction with tonic focusing of awareness, increased theta activity appears to reflect engagement of medial components of the attentional system, perhaps including the anterior cingulate gyrus. In contrast, the phasic attention required for scanning and updating the contents of working memory appears to be reflected in EP enhancement in lateralized regions of dorsalateral prefrontal cortex. Finally, the sustained effort required to maintain an inter-trial internal representation of the stimuli involves modulation of activity in widespread regions of parietal cortex, and appears to be reflected both in the stimulus-locked enhancement of a posterior slow potential, and in a tonic reduction in the amplitude of the alpha rhythm. This research was supported by AFOSR, NIMH, & ONR.

779.4

COMBINED PET AND ERP STUDY OF SUSTAINED VISUAL SPATIAL ATTENTION AND VISUAL TARGET DETECTION. M.G.Woldorff*, P.T.Fox, M.Matzke, S.Veeraswamy, J.L.Lancaster, P.A.Jerabek, C.C.Martin. Research Imaging Center, Univ. of Texas Health Science Center., San Antonio, TX 78284-6240.

PET studies have indicated various brain regions involved in visual attention, but they do not provide timing information and have tended not to distinguish between sustained attention and target-related processing/attention. ERPs have differentiated between, and revealed timing of, sustained-attention vs. target-processing activation changes, but precisely specifying the sources of these effects has been difficult.

In this project, ERPs and PET scans were both recorded (separate sessions) while subjects (n=9) fixated on a small cross and *bilateral lower visual-field stimuli* were rapidly presented (~2/sec). In active runs, subjects attended covertly to a designated side to detect target stimuli on that side. There were five conditions: (1) Attend right, many targets (16%); (2) Attend left, many targets; (3) Attend right, few targets (2%); (4) Attend left, few targets; (5) Passive viewing (2% irrelevant targets). Each subject had two PET runs (15-O water-bolus) and eight ERP runs (64-chan) of each condition. PET images were analyzed using Change Distribution Analysis after spatial normalization to Talairach space.

ERPs showed enhanced positivity from 80-150 msec over *contral. occipit. areas* for both targets and nontargets in active attention conditions vs. passive. This early-positivity attention effect did not differ between many- and few-target conditions, indicating similar sustained attention. Targets in active conditions also elicited large P300 waves (320-600 msec), preceded by contral. N2b's (250-300 msec). In the PET, active attention vs. passive conditions showed strong activity in *contral. dorsal occipit. areas* (BA18/19), which also was similar in the many- and few-target conditions. In conjunction with Heinze et al. (*Nature* 1994, 372: 543-546), which found attention effects in human contral. fusiform (but *not* dorsal occipit.) for *upper* visual-field stimuli, the present results suggest *human area V4 for lower visual field* may be in dorsal occipit. cortex and a source of the early ERP attention effect. Some activation was also seen in contral. fusiform in active vs. passive conditions, which may reflect activation of a ventral parallel pathway for object analysis. The *ant. cingulate* was very active in *both the many- and few-target conditions* vs. passive, with little difference between many and few, suggesting this area is not specific to "selection for action" *per se*. *No* focal blood-flow effect was apparent as a likely *P300* source, although some indication of distributed additional activity in the many-target conditions was seen in the overall population statistics. This combined PET/ERP study indicates multiple areas activated during visual attention and provides insight into their timing and functions.

779.5

CHANGES IN AUDITORY CORTEX ACTIVATION DURING SELECTIVE ATTENTION. C.L. Grady*, J.W. VanMeter, J.M. Maisog, P. Pietrini, J. Krasuski, J.P. Rauschecker. Lab. of Neuroscience, NIA, Lab. of Psychology and Psychopathology, NIMH, Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

Previous studies have shown that attention to a particular stimulus can increase the activity of the brain area responsible for processing that stimulus. We investigated the effects of selective auditory attention in superior temporal cortex using functional MRI in 5 young subjects (3F, 2M, 26 ± 5 yrs). Echo planar images, using a 1.5 Tesla GE scanner, were obtained while subjects listened to 3 word lists. Subjects heard each list three times (for a total of 9 scans), and were instructed to count the number of times they heard a particular word during two of these presentations. For the third, they listened to the words without counting. Each scan consisted of three periods of words alternating with three periods of no stimulation, each 30 sec in length, and the order of presentation of the word lists was varied across subjects. Images obtained during stimulation were compared to those obtained during rest using an ANOVA ($p<0.01$) with a subsequent test of significance on the spatial extent of each cluster of activated voxels ($p<0.01$). All subjects showed significant areas of activation in left auditory cortex during all listening conditions compared to rest. Right hemisphere activation also was seen in 2 subjects scanned with a full-head coil. Three of the five subjects showed a mean activation during the attention conditions that was substantially larger in spatial extent than that seen during the passive listenting condition (112%, 324% and 338%). Two subjects had an equivalent number of voxels activated in the attention and passive conditions. These results provide preliminary evidence in support of an attention related enhancement of activation in auditory cortex.

779.6

A LARGE-SCALE NEURAL MODEL LINKING LOCAL NEURONAL DYNAMICS TO POSITRON EMISSION TOMOGRAPHY (PET)-REGIONAL CEREBRAL BLOOD FLOW (rCBF) DATA. M-A. Tagamets[1], B. Horwitz[1] and J. A. Reggia*[2], [1]Lab. Neurosci, NIA, NIH, Bethesda, MD, 20892 and [2]Dept. Computer Sci., Univ. Maryland, College Park, MD 20742.

In order to better understand the neural mechanisms that underlie cognitive behavior, we have developed a large-scale neural model that relates local neuronal dynamics to functional neuroimaging data [e.g., regional cerebral blood flow (rCBF)] acquired by techniques such as positron emission tomography (PET) during performance of specific visual tasks. Distinct modules of the model represent identified visual cortical areas associated with the processing of information about color and shape. The model has two general properties: 1) fast local dynamics are modeled by explicit pre- and post-synaptic processes that modulate effective connection strengths, enabling discrimination and binding of features (e.g., color, shape) presented to a module representing primary visual cortex; (2) rCBF data are modeled by integrating over time the synaptic and neuronal activities generated by the model for each cortical area. We used this model to simulate a PET study of selective attention (color vs. shape), and showed that the task can be performed using spatiotemporal encodings similar to those suggested by electrophysiological results (e.g., Singer, Ann. Rev. Neurosci., 1993). The importance of a "top-down" effect on task performance is demonstrated. We also assessed the implications of fast temporal feature binding for interpreting PET data by relating the fast local dynamics to both region-specific rCBF activations and interregional rCBF correlations.

779.7

A PET STUDY OF AUDITORY AND VISUAL ATTENTION
D.S. O'Leary,* N.C. Andreasen, R. Hurtig, L. Flashman, I. Torres, R. Hichwa, Mental Health Clinical Research Center, University of Iowa, Iowa City, IA 52242.

We have previously found that attending right or left for dichotically-presented stimuli caused asymmetric changes in rCBF in auditory cortices (O'Leary et al., *Brain and Language*, in press). The present study used simultaneously-presented visual and auditory stimuli to assess the modality-specificity of spatial attention. During different conditions of a PET study with (O-15) water, 13 normal volunteers attended left and right to visually- or auditory-presented consonant-vowel-consonants (CVCs). During visual conditions, subjects fixated centrally, but monitored left or right visual fields while ignoring dichotic stimuli. Attention to one or the other visual fields caused asymmetric rCBF changes in extrastriate cortices, but did not induce asymmetric rCBF changes in auditory cortices. The activation in auditory cortices resulting from nonattended dichotic stimuli was symmetrical. When subjects attended to dichotic stimuli, we replicated our finding that attending left or right caused asymmetric rCBF in temporal lobes, but found that rCBF in occipital cortex was unaffected. The pattern of rCBF change indicates that spatial attention is modality-specific. Regions of interest were traced on each individual's MR images which were co-registered with their PET images, allowing measurement of rCBF in Heschl's gyrus, the planum temporale, the ascending ramus of the sylvian fissure and striate cortex. These data, which will be discussed, address the anatomical location at which attentional effects occur in sensory sytems (i.e., primary vs secondary cortices).

779.8

ATTENTION MODULATES fMRI ACTIVATION IN HUMAN MT/MST. K.M. O'Craven[1,2], R.L. Savoy[1,2]*, B.R. Rosen[2]. [1]*The Rowland Institute for Science, 100 Edwin H. Land Blvd, Cambridge, MA, 02142.* [2]*MGH-NMR Center, 149 13th St., Charlestown, MA 02129* (kmo@nmr.mgh.harvard.edu)

We used functional MRI to measure the amount of activation within functionally defined regions of the human brain homologous to monkey areas MT and MST. Subjects viewed a single stimulus that consisted of both moving and stationary dots. Motion was radial, and subjects fixated a central fixation point. Initially, subjects attended to the moving dots and ignored the stationary dots. Every 20 seconds, a verbal cue instructed subjects to voluntarily switch attention between the moving dots and the stationary dots. The magnitude of the fMRI signal was significantly higher while subjects attended to the moving dots than while they attended to stationary dots and ignored the moving dots.

An additional experiment sought to quantify more precisely the modulation just described. Subjects viewed the moving-and-stationary-dot stimulus during four 20 second epochs, alternating with epochs of a stimulus in which only stationary dots were present. During two of the four epochs of moving-and-stationary-dots, subjects attended to the moving dots, and during the other two epochs, they attended to the stationary dots. Attending to stationary dots did not eliminate MT activation. Rather, MT was highly active any time a stimulus containing visual motion was present. However, when attention was directed to the stationary dots, activity was only 80-90% as strong as when attention was directed to the moving dots. This attentional modulation was significant ($p < .001$) for each of three subjects.

What does the modulation of MT activation represent? Is it *additional* activation during the "attend moving" condition, or is it *suppressed* activation during the "attend stationary" condition, because the moving dots need to be ignored? Experiments are underway to examine this question.

779.9

FUNCTIONAL MRI OF AUDITORY EFFORTFUL ATTENTION IN HUMANS. HC Breiter, LJ Seidman, JM Goodman, JM Goldstein, KM O'Craven, RM Weisskoff PWR Woodruff, R Savoy, A Jiang, D Kennedy, W Kennedy, MT Tsuang, BR Rosen*. Harvard Medical School and MGH-NMR Center, Charlestown, MA 02129.

Attention becomes effortful with increased working memory load, needs for interference control, or difficulty of target detection. Using functional MRI (fMRI), we investigated if an auditory effortful attention task would activate regions reported for visual selective attention, including brainstem and thalamus. Seven normal men had contiguous 7mm axial images covering pons to parietal cortex acquired with an asymmetric spin-echo instascan sequence. The paradigm employed an A-B-A-B design with stimulus blocks of 90 letters presented aurally one letter per second. In A, subjects responded to the letter "a" when immediately preceded by a "q" (QA). B conditions required the subject to respond to the letter "a" when preceded by a "q" four letters previously (Q3A), and ignore embedded false cues and targets. Target probability was matched between QA and Q3A conditions at 20%. Subjects were scanned three times. FMRI time series data was transformed into Talairach space and evaluated using three novel averaging techniques and nonparametric statistical mapping. Compared to QA, the Q3A condition produced, at the Bonferroni level ($p<10^{-7}$), positive change bilaterally in the parietal-occipital cortex (PPC), thalamus, superior colliculus (SC), supplementary motor area (SMA), frontal eye fields (FEF) and dorsolateral prefrontal cortex, and unilaterally in right inferior temporal cortex (ITC). Significant negative signal change occurred in the anterior cingulate (AC). This study found activation in regions proposed by Posner and Peterson to form a posterior network for orienting to sensory events (ie, PPC, thalamus, and SC), along with regions forming an anterior network for signal detection (ie, AC, SMA). Our auditory task also produced activation in visual regions such as PPC, SC, ITC and FEF; these results may relate to cognitive strategy employed. Effortful attention appears to depend on the entire distributed network implicated for selective attention, with important components being independent of sensory modality of stimulus.

779.10

rCBF IN CINGULATE, FRONTAL, AND PARIETAL CORTICES IS HIGHLY CORRELATED WITH ACCURATE DECISION RESPONSE TIME DURING TONE RECOGNITION. H.H. Holcomb*, P.J. Caudill, Z. Zhao, D.Medoff, H.T. Ravert, R.F. Dannals, C.A. Tamminga. Maryland Psych. Res. Ctr. and Johns Hopkins Med. Instit., Baltimore, MD 21228.

When trying to recognize one of two similar stimuli a subject must suppress irrelevant and distracting information, and enhance his attention to important features which help him distinguish one from another. The cingulate cortex appears to play an important role in decision physiology. We have used an auditory recognition task in conjunction with the 15-oxygen, bolus water, PET rCBF method to further understand the role of various cortical regions in making difficult auditory recognition decisions. Twelve normal volunteers were trained (3200 trials) to recognize high frequency versus low frequency tones in a forced choice recognition task. Twelve rCBF scans were obtained from each subject: 4 resting, 4 sensory-motor controlled, and 4 decision. The average accuracy for the 60 trials presented during each decision scan was 80%, median accurate response time = 683 msec. Data were registered (Woods et al, 1992) and stereotaxically normalized (Friston et al, 1995). Statistical Parametric Mapping (SPM94, Hammersmith Cyclotron Unit) was used in conjunction with other correlation matrix algorithms (Horwitz and Rapoport, 1988) to assess rCBF relationships with accurate trial response times (RT). Four regions exhibited highly significant positive correlations ($r >0.5$, $p<0.001$) with RT during decision scans: anterior cingulate, mid - frontal right, mid - frontal left, and inferior parietal lobule left. The cingulate cortex also exhibited highly significant correlations with each of the other three regions ($p < 0.001$). These associations suggest that the longer it takes a subject to make his decision the greater will be his blood flow rate to the anterior cingulate, midfrontal and left parietal cortices.

779.11

REFLEX SACCADES IN PATIENTS WITH UNILATERAL VISUAL EXTINCTION. C.A. Marzi*, A. Fanini, G. Gambina, A. Ipata, C. Miniussi, N. Smania, G. Tomelleri. Dept. of Neurological & Visual Sci., Univ. of Verona, and Neurology Div. Ospedale Borgo Trento, Verona, Italy.

Patients with unilateral left visual extinction as a consequence of right hemisphere damage centered on the parietal lobe were tested on a simple manual reaction time task with lateralized brief flashes. There were three conditions of stimulus presentation: unilateral left and right hemifield stimuli and bilateral stimuli across the vertical meridian. Ss were instructed to keep their gaze steady onto a central fixation point both before and after stimulus presentation. Following execution of the response, the patients were asked to report on the number of stimuli just seen. In spite of the instructions, there was an overall mean of about 25% reflex-like, stimulus driven, saccades following either left or right unilateral stimuli. With bilateral stimuli about two/thirds of saccades were directed to the ipsilesional hemifield. The latency peak of saccades to unilateral right stimuli was shorter (162.5 msec) than that for saccades to left stimuli (237 msec) but showed the same latency as that for right saccades following bilateral stimuli. The lack of left-directed saccades with bilateral stimuli was present in both extinguished and correct trials. The same was true for the lack of interference of the left stimuli on saccadic latency to bilateral targets. The present results suggest that the simultaneous presentation of ipsilesional stimuli has a profound inhibitory effect on reflex saccades elicited by contralesional stimuli. However, such an impairment does not seem to be related to the perceptual phenomenon of extinction.

NEUROTROPHIC FACTORS: RECEPTORS AND CELLULAR MECHANISMS V

780.1

SEQUENTIAL EXPRESSION OF TRKS A, B AND C DURING DEVELOPMENT AND REGENERATION OF OLFACTORY RECEPTOR NEURONS A. Jane I. Roskams*, Angelyn Bethel, Laurie Williams and Gabriele V. Ronnett. Department of Neuroscience and Neurology, Johns Hopkins School of Medicine, 725 N. Wolfe Street, Baltimore MD 21205.

Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins -3,4 and 5 (NT-3,4,5) have been identified and characterized by their ability to induce neuronal differentiation and support neuronal survival in vivo and in vitro. A family of neurotrophin receptor tyrosine kinases (Trks) mediates neurotrophin action and neurotrophin binding has been shown to stimulate homodimerization of one Trk, heterodimerization of two Trks or one Trk and the low affinity NGF receptor (LNGFR). Trk A,B,C and the lNGR are expressed in adult and developing olfactory neuroepithelium. The olfactory neuronal epithelium is capable of undergoing neuronal regeneration throughout the lifetime of the organism. When the olfactory bulb is removed, the neuroepithelium enters a state of co-ordinate regeneration, where there is a sequential expression of Trk A (precursor neurons), B (immature neurons) and C (mature neurons) within regenerating olfactory neurons at different stages of differentiation. In primary cultures of olfactory neurons Trks become specifically phosphorylated when challenged with the appropriate neurotrophin ligand and activate a signal transduction cascade that terminates in the up-regulation of immediate early genes such as fos and jun. We are currently examining the olfactory pathways of mice with a null mutation for the Trk A, B and C genes to determine whether the phenotype of these knockout mice supports the suggested in vivo role for each Trk and to examine the possibility of neurotrophin receptor redundancy during development in this system.

780.2

THE EXTRACELLULAR ACIDIFICATION RESPONSE OF TRK A EXPRESSING CELLS TO NGF IS DOWN-REGULATED BY CO-EXPRESSION OF P75 IN PC12 CELLS AND TRANSFECTED L CELLS. H. G. Wada^, J. Twiss†, G.T. Baxter*^, K.S. Fok^, E.M. Shooter†, and H. H. Sussman†. ^Molecular Devices Corp., Sunnyvale, CA 94089, †Stanford University, Stanford, CA 94305.

Neurotrophins play a central role in neuronal development and maintenance. These molecules exist primarily as dimers and bind to a family of neurotropin receptors, which include trkA, trkB, and trkC. Another receptor for neurotropins is $p75^{NGFR}$ which is a member of the TNF receptor family. We have used the Cytosensor® microphysiometer to study the role of p75 in combination with trkA, the putative high affinity receptor for NGF. The Cytosensor measures increases in extracellular acidification rate in response to receptor activation of intracellular signalling pathways. In one set of experiments, PC12 cells transfected with anti-sense sequences directed against either trkA or p75 were tested with wild type PC12 cells as controls. Anti-sense-trkA cells had a suppressed acidification response to 10 ng/ml NGF compared to the wild type PC12 cells, and anti-sense-p75 cells had an enhanced response to the same amount of NGF. It was also noted that an anti-p75 monoclonal antibody (MC192) which does not inhibit NGF binding also suppressed NGF responses in wild type PC12 cells. These data suggest that p75 has a negative regulatory function for acidification responses to NGF in PC12 cells. A similar result was obtained using L cells transfected either with trkA alone or with both trkA and p75. The trkA transfected cells gave large (80%) increases in acidification rate which were sustained over the time of neurotropin exposure, and the trkA/p75 transfected cells gave an attenuated response (15%) which rapidly returned to baseline, during the neurotropin exposure. These results suggest that p75 may have an inhibitory or desensitizing effect on trkA activation by NGF. This study was supported in part by Defense Advance Research Projects Agency, Contract MDA972-92-C-0005.

780.3

DYNAMICS OF *TRKA* PHOSPHORYLATION IN RESPONSE TO INTRACEREBRAL NGF INJECTIONS IN RODENTS. L.F. Kromer,* and D.R. Kaplan[1]. Dept. of Cell Biology, Georgetown University Medical Center, Washington, DC 20007 and [1]the National Cancer Institute, Frederick, MD 21702

Cholinergic neurons in both the septum/basal forebrain and neostriatum are responsive to nerve growth factor (NGF) and express *trkA* receptors, the high affinity receptor for NGF. Although PC12 cells have been used extensively to study NGF induced *trkA* phosphorylation and subsequent signal transduction *in vitro*, little is currently known about NGF induced signaling through *trkA* receptors *in vivo*. Thus, the present experiments were designed to evaluate the dose response and time course of NGF induced *trkA* phosphorylation in both the septum/diagonal band (S/DB) and neostriatum (NS) after intraventricular (ICV) injections of NGF in adult rats. ICV injections of NGF exhibit a clear dose response (10ng, 100ng, and 1μg) in the degree of receptor phosphorylation in both the S/DB and NS. Moreover, a single ICV injection of NGF (1 μg/2-4 μl PBS) results in a prolonged receptor activation in the S/DB and NS that can be detected by 30 min., is maximal from 2-12 hrs., and decreases to baseline levels by 24-36 hrs.. To determine whether the dynamics of this response could be altered, multiple ICV injections of NGF were administered. When 3 ICV injections of NGF are administered at 48 hr. intervals, there is a prolonged activation of *trkA* receptors in both the S/DB and NS which lasts for at least 72 hrs. This potentiation of receptor phosphorylation with multiple NGF injections has important implications for the design of possible CNS treatment therapies with neurotrophic factors. Supported by NIH grant NS-31445.

780.4

REDUCTION AND LOSS OF INNER EAR INNERVATION IN *trk*B AND *trk*C RECEPTOR KNOCK OUT MICE. B. Fritzsch*, @A. M. Fagan, #R. Smeyne, and @I. Silos-Santiago Creighton Univ., Omaha, NE 68104, @Bristol-Myers Squibb, Princeton, NJ 08543, and #Roche Inst. Molec. Biol., Nutley, NJ 07110.

The pattern of afferent and efferent innervation of the ear was investigated in embryonic and neonatal, aldehyde fixed mice homozygotic for the *trk*B or *trk*C gene deletions using DiI. $trkB^{-/-}$ mice developed innervation to all sensory epithelia by 12.5 dpc but lost it to the semicircular canals by 13.5 dpc. The utricle and saccule showed a reduction in both afferent and efferent innervation. Efferent innervation to the vestibular region developed delayed and only to areas with an afferent innervation. $trkC^{-/-}$ mice had fewer spiral ganglion cells in the cochlear basal turn. $trkB^{-/-}/trkC^{-/-}$ double knock out mice had no innervation at all by $P0_{+/-}$. Mice homozygotic for $trkB^{-/-}$ and heterozygotic for $trkC^{+/-}$ displayed the $trkB^{-/-}$ vestibular phenotype but showed only a patchy innervation of the cochlea by very few spiral ganglion cells. In contrast, mice heterozygotic for $trkB^{+/-}$ and homozygotic for $trkC^{-/-}$ showed only the phenotype of a simple $trkC^{-/-}$ mutant. These data show that TrkB and TrkC receptors are both necessary and sufficient for otic ganglion cell survival. Supported by NIH (P50 DC00215-09).

780.5

IDENTIFICATION OF A NERVE GROWTH FACTOR RECEPTOR COMPLEX BY BIOPHYSICAL APPROACHES. A.H. Ross*, C.A. McKinnon, M.-C. Daou, R.M. Stephens, D.R. Kaplan and D.E. Wolf. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545 and NCI-Frederick Cancer Research and Development Center, Gaithersburg, MD 21701.

It has been proposed that the functional high-affinity nerve growth factor receptor is a heterodimer of two proteins gp75 and TrkA. We have obtained direct evidence of the existence of this complex by a combined approach of fluorescence recovery after photobleaching (FRAP), copatching fluorescence confocal microscopy, and site-directed mutagenesis. When gp75 is expressed alone in either mammalian PC12 cells or using baculovirus-infected insect Sf9 cells, it is highly mobile as determined by FRAP. Coexpression with TrkA causes a reduction in the mobile fraction. TrkA immobilization of gp75 is not observed, if the cytoplasmic domain of gp75 is truncated or if TrkA is mutated to inactivate the tyr-kinase domain. Analysis of diffusion coefficients in the presence and absence of NGF suggests that gp75 and TrkA are complexed, even for mutated receptors which do not bind NGF with high-affinity. Direct evidence of gp75-TrkA complex was obtained by copatching studies. It was found that patching of gp75 by crosslinking antibodies resulted in copatching of TrkA. Analysis of mutated receptors by copatching indicates receptor complexes in the absence of high-affinity NGF binding sites. Using chimeric receptors of TrkA and Tor, we determined that the extracellular domain of TrkA is critical for efficient copatching. We suggest that extracellular interactions drive complex formation, but functional high-affinity NGF binding results from a conformational change involving intracellular and extracellular domains of both receptors.

780.6

DIFFERENTIAL DEVELOPMENTAL EXPRESSION OF THE NEUROTROPHIN-3 RECEPTOR (TrkC) ISOFORMS IN THE ENTERIC NERVOUS SYSTEM. **J.D. Lewis, C.J. Davidson, J.K. Davies and L.M. Kaplan.*** Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA 02114.

cDNA cloning studies have suggested the existence of multiple, neurotrophin-3 (NT-3) receptor isoforms encoded by the *trk*C gene. In the rat, alternative splicing of the primary *trk*C transcript generates four "kinase competent" isoforms that encode receptors varying by the presence or absence of 14, 25, or 39 amino acid inserts in the tyrosine kinase domain, respectively (C_0, C_{14}, C_{25}, and C_{39}). Four additional "truncated" TrkC isoforms, with identical extracellular ligand-binding domains, contain unique intracellular sequences in place of the kinase domain (TTC_{108}, TTC_{113}, TTC_{143}, and TTC_{158}). We have developed two reverse transcription polymerase chain reaction (RT-PCR) based strategies to measure specific expression of the various *trk*C isoforms. First, quantitative PCR with an easily detected, competitive mutant construct is used to quantitate total *trk*C expression. Primers flanking the kinase domain splice site competitively amplify the four kinase competent isoforms, allowing measurement of the relative expression of these mRNAs. The second assay employs similar techniques to measure expression of mRNAs encoding the truncated receptors. These studies reveal that C_0 and C_{14} are the predominant kinase competent isoforms and TTC_{158} is the predominant truncated isoform in peripheral tissues and within the gastrointestinal tract. C_{25}, C_{39}, TTC_{108},and TTC_{113} are expressed at lower levels in selected tissues. Isoform expression is strongly regulated during late stages of ENS development with selective increases in the expression of the C_{25}, C_{39} and TTC isoforms. The regulated expression of these functionally distinct isoforms suggests that response to NT-3 is determined by the developmental and physiologic state of the target neuron.

780.7

ENHANCED EXPRESSION OF trk B mRNA AND Trk B PROTEIN FOLLOWING FOREBRAIN ISCHEMIA OF RAT BRAIN K.Iihara, T. Tsukahara*, N. Hashimoto, T. Okubo, T. Taniguchi, Dept. of Cerebrovascular Surg., National Cardiovascular Center, Osaka 565; Dept. of Neurobiology, Kyoto Pharmaceutical Univ., Kyoto 606; Japan

We have shown that brain-derived growth factor (BDNF) ameliorates delayed neuronal death in the hippocampus after forebrain ischemia of rat brain. Here, we examined the expression of trk B mRNA and Trk B protein following forebrain ischemia of rat brain. Transient forebrain ischemia was induced on male Wistar rats according to the method reported by Smith et al. Northern blot analysis revealed that ischemia markedly increased the expression of trk B mRNA in the hippocampus with a peak at 4h post ischemia. In situ hybridization revealed that increased mRNA expression in the hippocampus was observed mainly in pyramidal neurons in the CA1 sector and in granular neurons in the dentate gyrus with a peak at 4h. Western blot analysis indicated that the expression of $gp^{145trkB}$ in the hippocampus increased from 30 min to 6h with a peak at 4h post ischemia. Immunohistochemistry disclosed an enhanced immunostaining for trk B protein in pyramidal neurons in the CA1 sector and in granular neurons in the dentate gyrus with a peak at 4h. Thereafter, this increased immunostaining rapidly declined, but was still moderately enhanced at 24h post ischemia. These findings revealed that forebrain ischemia markedly increases the expresssion of trk B at both mRNA and Trk B protein level especially in the hippocampus, suggesting a crucial and local role of BDNF/trk B system in the forebrain ischemia of rat brain.

780.8

ZEBRAFISH TRK RECEPTOR EMBRYONIC EXPRESSION AND LIGAND SPECIFICITY. S. C. Martin*[1], M. Hashimoto[1], R. Götz[3], J. H. Sandell[2] and G. Heinrich[1] [1]Section of Biomolecular Medicine, [2]Dept. of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118. [3]Dept. of Neurology, University of Würzburg, Würzburg 97080, Germany.

We have isolated five Trk receptors from the zebrafish (Martin *et al.*, 1995 Dev. Biol., in press). The ligand specificities of these receptors has been investigated by analysis of phosphotyrosine induction in response to the neurotrophins. Each of the human neurotrophins: nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4 were tested in this assay with four of the zebrafish Trk receptors. One Trk receptor responded only to human NT-3, another only to BDNF. Experiments are underway to examine the specificity of the other Trk receptors with human and *Xiphophorous* neurotrophins.

The onset of expression of two of the zebrafish Trk receptors was examined by whole mount *in situ* hybridization. The NT-3 responsive Trk receptor was detected by *in situ* hybridization at 24 hours after fertilization in the hindbrain. In some embryos there were a total of six positive cells arranged as two rows of three cells, while in other embryos a total of 10 positive cells were found. The expression of another zebrafish Trk receptor, whose ligand has not been identified, was detected 16 hours after fertilization. A total of approximately 100 positive cells were detected in the cranial ganglia, tail and forebrain. Double labeling techniques, with markers for known cell types, are being used to investigate the identity of the positive cells. The distinct expression pattern and ligand specificities for these zebrafish Trk receptors suggests separate roles in the zebrafish nervous system. (R01-NS22422 to G.H.)

780.9

CO-LOCALIZATION OF EPH-RELATED RECEPTOR TYROSINE KINASES AND THEIR LIGANDS IN THE DEVELOPING NERVOUS SYSTEM. J.A. Holash*(1), E. Magal (2), R.A. Lindberg (2), R.J. Toso (2), D. Chang (2), H. Shao (3), V. Dixit (3) and E.B. Pasquale (1). 1) La Jolla Cancer Res. Foundation, La Jolla, CA 92037. 2) AMGEN Inc., Thousand Oaks, CA 91320. 3) Dept. of Pathology, Univ. of Michigan Med. Sch., Ann Arbor, MI 48109.

Members of the Eph-subfamily of receptor-protein tyrosine kinases are highly expressed in developing nervous tissue. This suggests that they are important in regulating the survival and differentiation of neural cells. The identification of ligands for Eph-related receptors is facilitating the investigation of their function. The relevance of receptor-ligand pairs in vivo can be assessed by determining where a receptor and its ligand are co-localized. Hypotheses regarding the receptor-ligand pair function can then be made. We have found, using immunohistochemistry, that Eck and its ligand, B61, are co-expressed in the developing rat spinal cord region. This prompted us to examine the effects of B61 on cultured spinal cord neurons. In these assays, B61, by acting through the receptor Eck, enhances neuronal survival and neurite outgrowth (Lindberg et al., CSH Symposium, 1995). In addition to the spinal cord region, B61 and Eck have overlapping patterns of distribution in the brain, as well as some cranial nerves; therefore, B61 and Eck may be active in a number of other nervous system structures. Interestingly, while the expression of Eck appears to be restricted to neuronal processes, B61 has a more diffuse pattern of expression. Because B61 is a secreted protein, it is possible that its site of production differs from the site where it is ultimately localized. We are currently immunostaining cultured neural cells to determine the cellular origin of B61 in nervous tissue.

We are also comparing the distribution of the Eph-related kinase Cek5, which is expressed in the inner plexiform and ganglion cell layers of the retina, with that of the Cek5 ligand by using both immunohistochemistry and a Cek5-Ig chimera. Our preliminary results indicate that the Cek5 ligand is co-expressed with Cek5 in the embryonic day 11 chicken retina. This suggests that Cek5 and its ligand are likely to function in the development of the visual system.

780.10

bFGF AND FGF RECEPTOR ACCUMULATE IN CELL NUCLEUS IN ASSOCIATION WITH CELL PROLIFERATION - NOVEL MECHANISM FOR CELL CYCLE REGULATION. **M.K. Stachowiak, E. Mordechai, A. Joy, K. Neary, R. Fischer*, P. Maher*, R. Florkiewicz*, E.K. Stachowiak.** Barrow Neurol. Inst., Phoenix, AZ 85013,* The Scripps Res. Inst., La Jolla CA 92037.

Sections of human brain stained with bFGF antibodies revealed the presence of bFGF in the nuclei of reactive astrocytes of the epileptic foci and in neoplastic astrocytes of glioma tumors. The mechanisms controlling induction and function of nuclear bFGF were studied in vitro. Confluent human astrocytes were quiescent and did not express nuclear bFGF. Following passage, subconfluent astrocytes re-entered cell cycle and nuclear accumulation of bFGF was observed. Glioma cells expressed nuclear bFGF and proliferated continuously independent of cell density. In an effort to determine how intracellular bFGF exerts its biological effects we discovered high affinity (kd=15 pM) ^{125}I-bFGF binding sites in the isolated nuclei. Western analysis confirmed that FGFR1 is located predominantly in the nuclei and that cytoplasm contains only trace their amounts. Intranuclear localization of FGFR1 was demonstrated by confocal microscopy. In astrocytes, the levels of nuclear FGFR1 are coregulated with bFGF in association with changes in cell density and proliferation. Glioma cells expressed nuclear FGFR1 constitutively. Glioma cell lines refractive to stimulation with exogenous bFGF, proliferated faster in response to intracellularly expressed bFGF. Increased cell proliferation coincided with nuclear accumulation of bFGF and was observed in cells expressing nuclear FGFR1, but not in cells that express only cytoplasmic FGFR1. We propose that transient nuclear accumulation of bFGF and FGFR1 proteins may promote reversible mitotic activation of quiescent human astrocytes. Deregulation of bFGF and FGFR1 expression and constitutively elevated nuclear bFGF and FGFR1 may contribute to the uncontrolled growth of glioma cells (NSF, NIH, APDA).

780.11

EXPRESSION AND DISTINCTIVE PHOSPHORYLATION OF THE CEK9 RECEPTOR TYROSINE KINASE IN THE DEVELOPING CHICKEN EMBRYO. Chandrasen Soans, Jocelyn A. Holash, Yelena Pavlova and Elena B. Pasquale*. La Jolla Cancer Research Foundation. 10901 N. Torrey Pines Road, La Jolla, CA 92037

Cek9 is a receptor tyrosine kinase of the Eph subfamily for which only a partial cDNA sequence was known (Sajjadi and Pasquale, 1993). We have obtained the entire cDNA sequence and identified a variant form of Cek9 that lacks a signal peptide. We subsequently examined the spatio-temporal expression and tyrosine phosphorylation of Cek9 during the development of the chicken embryo. Cek9 expression can be detected in many tissues and is particularly prominent in neural tissues like the cerebrum, cerebellum and retina. Immunohistochemistry reveals that Cek9 is concentrated in areas containing neuronal processes, such as the inner plexiform layer and nerve fiber layer of the retina, and the molecular layer of the cerebellum. Unlike other Eph-related kinases, Cek9 is substantially phosphorylated on tyrosine in many embryonic tissues at various developmental stages. Since we have shown that autophosphorylation of Cek9 correlates with increased *in vitro* enzymatic activity, our results suggest that Cek9 plays a role in signal transduction pathways that are active during neural development. Tyr 796, which is located in the catalytic domain of Cek9, corresponds to a tyrosine residue that is highly conserved in both cytoplasmic and receptor tyrosine kinases. The mutation of Tyr 796 to Phe leads to a dramatic reduction in the autophosphorylation of a GST-Cek9 fusion protein expressed in bacteria. Thus, Tyr 796 is important for autophosphorylation activity and/or is the major site of Cek9 autophosphorylation. (This work was supported by NIH grant HD 26351).

780.12

INSULIN INHIBITS p38 MITOGEN-ACTIVATED PROTEIN (MAP) KINASE IN CULTURED FETAL NEURONS. J.L. Kummer and K.A. Heidenreich*, Department of Pharmacology, UCHSC, and the Denver VAMC, Denver, CO 80262

The MAP kinase cascade involving Erk1/2 is a major signalling system by which cells transduce extracellular stimuli into intracellular responses. Recently, 2 other MAP kinase cascades, involving JNK/SAPK and p38/RK, have been discovered. Little is known about the regulation of these MAP kinases by neurotrophic factors. In this study, we examined the MAP kinases present in fetal chick forebrain neurons and their regulation by insulin, a potent neurotrophin for these cells. Neuronal cell lysates chromatographed on Mono Q and assayed with myelin basic protein (MBP) showed 3 peaks of protein kinase activity eluting at 30 mM NaCl (Peak 1), 170 mM NaCl (Peak 2), and 420 mM NaCl (Peak 3). Peak 1 contained JNK (assayed with a GST-c-jun fusion protein) and peak 2 contained ERK1/ERK2 (identified with ERK1/ERK2 C-terminal antibodies). Insulin (50 ng/ml, 15 min) had no significant effect on JNK or ERK1/ERK2 activities but markedly inhibited the 3rd peak of kinase activity by 80-90%. Analysis of Peak 3 by MBP-impregnated gel assays revealed a 38 kDa kinase. Like other MAP kinases, the p38 MAP kinase bound to phenyl-sepharose. However, the p38 MAP kinase was distinct from ERK1/ERK2 and JNK since it had a lower molecular weight, eluted at higher salt concentrations, and had distinct substrate specificity. p38 did not significantly phosphorylate the EGF receptor peptide or GST-c-jun, but was highly active in phosphorylating the transcription factor ATF-2. These characteristics are typical of the p38/RK kinases. In summary, insulin had no significant effect on JNK or ERK1/ERK2 MAP kinases in fetal neurons, but markedly inhibited p38 MAP kinase in these cells. Since p38 has been linked to cell apoptosis, the inhibition of this kinase by insulin may represent a mechanism by which insulin increases neuronal survival.

EXCITATORY AMINO ACID RECEPTORS XIII

781.1

MEMBRANE-DELIMITED MODULATION OF NMDA RECEPTOR CHANNELS BY METABOTROPIC GLUTAMATE RECEPTORS VIA PERTUSIS TOXIN-INSENSITIVE G PROTEINS ON MOUSE CORTICAL NEURONS. S.P. Yu*, D.M. Turetsky and D.W. Choi. Department of Neurology and Center for the Study of Nervous System Injury, Washington Univ. School of Med., St. Louis, MO 63110.

The excitatory neurotransmitter glutamate activates both ionotropic and metabotropic receptors (mGluRs). mGluR activation modulates many neuronal mechanisms, including the behavior of ionotropic NMDA or AMPA/kainate receptors, usually by inducing changes in the levels of the second messengers, IP_3 or cAMP. Using whole cell and single channel recordings, we studied the modulation of NMDA receptor channels by mGluRs on mouse cortical neurons. The mGluR agonists 1S-3R-ACPD (200 μM), L-CCG-I (100 μM), (S)-3-hydroxyphenylglycine (3HPG, 100 μM) and (S)-4-carboxy-3-hydroxyphenyl-glycine (4C3HPG, 100 μM) attenuated NMDA-induced whole cell current by 20-30%. However, bath-applied 3HPG (mGluR 1/5 agonist) and 4C3HPG (mGluR 2/3 agonist) showed no effect on NMDA single channel activity in cell-attached recordings. In isolated outside-out patches, 20-100 μM 3HPG but not 4C3HPG decreased the open probability (P_{open}) of NMDA receptor channels. This modulation was blocked by the mGluR antagonist, (R,S)-α-methyl-4-carboxyphenylglycine (MCPG, 500 μM), as well as $GDP_\beta S$ (200 μM), but not by pertusis toxin (PTX, 0.2mg/ml for 20-24 h). 3HPG itself is unlikely a NMDA receptor antagonist since, in HEK 293 cells lacking endogenous mGluRs, it did not affect single channel currents mediated by cloned NMDAR1/2A receptors. Present results thus provide novel evidence that mGluR 1/5 may interact locally with NMDA receptors via PTX-insensitive G proteins, without the involvement of readily diffusible messengers. This modulation may have important implication for synaptic plasticity or neurotoxicity.
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781.2

DETERMINATION OF SUBUNIT COPY NUMBER OF RECOMBINANT NMDA RECEPTORS. P.Stern[2], P.Behe[2], M.Nassar[1], D.Wyllie[2], D. Colquhoun[2] and R.Schoepfer*[1,2] Laboratory for Molecular Pharmacology[1] and Dept. Pharmacology[2], University College London, WC 1E 6BT, UK.

Coexpression of wild-type and mutant subunits has been used to determine stoichiometry of neuronal nicotinic acetylcholine receptors, potassium channels and other multisubunit proteins.

We have explored whether mutant NMDA receptor subunit cDNAs expressed in *Xenopus* oocytes results in glutamate-gated channels with significantly altered single channel conductances. Coexpression of mutant N598R NR1 subunits together with wild-type NR2A subunits ($NR1^{mut}/NR2A^{wt}$) resulted in channels showing a main conductance of 2.6pS and a sublevel of 1.2pS (number of patches n=4). These are clearly distinct from the $NR1^{wt}/NR2A^{wt}$ channels which show a main conductance of 53.5pS with a sublevel of 42.3pS (n=3). Single channel conductances were determined by simultaneous least-squares fitting of the duration and amplitude of individual openings with a convolved step-response function.

Coexpression of $NR1^{mut}/NR1^{wt}/NR2A^{wt}$ subunits resulted in channels showing conductances of 15.2pS and 11.4pS (n=5) in addition to the ones found in $NR1^{wt}/NR2A^{wt}$ and $NR1^{mut}/NR2A^{wt}$ channels. These two conductances are linked by frequent direct transitions which suggests that they originate from the same channel type. This result indicates that there are at least two copies of the NR1 subunit present in one NMDA receptor molecule,

This approach to the determination of NMDA receptor stoichiometry is complicated by the need to measure very small conductances, as well as by the presence of subconductances.
Supported by The Wellcome Trust, Royal Society, HFSPO and MRC.

781.3

ARE IONOTROPIC GLUTAMATE RECEPTORS PENTAMERIC ASSEMBLIES? A.V. Ferrer-Montiel* and M. Montal. Dept. Biology. Univ. California San Diego. La Jolla. CA 92093.

Ionotropic glutamate receptors (iGluRs) are neurotransmitter-activated ion channels that mediate excitatory synaptic transmission in the CNS. These membrane receptors are composed of multiple subunits organized around a central pore. Their subunit stoichiometry remains elusive. In analogy to the nicotinic acetylcholine receptor (AChR), the prototype of ligand-gated ion channel, iGluRs are considered to be pentamers. Recent data, however, suggest that iGluRs and AChRs may have different protein topologies. This raises the question: are iGluRs pentameric assemblies? To address this issue we used a functional assay[1] based on the differential sensitivity of two GluR1 mutant receptor subunits to open channel blockers such as PCP and MK-801. Coinjection into *Xenopus* oocytes of weakly-sensitive subunits with highly-sensitive mutant subunits produced functional receptor channels with mixed drug sensitivities. Augmenting the fraction of the sensitive subunit in the mixture increases the affinity of the complex for the channel blockers. Assuming that both classes of mutant subunits aggregate randomly, the subunit stoichiometry of GluR1 was determined to be pentameric. This finding that iGluRs are pentamers is compatible with a conserved subunit stoichiometry for the members of the ligand-gated ion channel superfamily.

[1]MacKinnon, R. (1991). ***Nature 350***, 232-235. (Supported by NIH, DAMD and ONR)

781.4

DETERMINATION OF GLYCINE BINDING SITES IN THE M3-M4 LINKER OF THE NMDA RECEPTOR NR1 SUBUNIT. M.W. Wood, H.M.A. VanDongen, and A.M.J. VanDongen*. Dept. of Pharmacology, Duke Univ, Durham, NC 27710.

Initial topology models for ligand gated ion channels included four putative transmembrane domains (M1-M4). The ionotropic glutamate receptors are a subfamily of these channels for which the four transmembrane domain model has recently been rejected. NMDA receptors are a glutamate receptor subtype comprised of two subunits (NR1 and NR2). By manipulating N-glycosylation sites in the NR1 subunit, we have localized the extreme portions of the M3-M4 linker to the extracellular space. Introduction of a novel glycosylation site immediately past M3 resulted in a functional channel displaying a fractional response to glutamate, containing only contaminating amounts of the coagonist glycine. Attempts to map the M3-M4 linker region in other glutamate receptors have resulted in non-functional mutants. Despite the inability to functionally verify proper protein folding, it has been concluded that the entire M3-M4 linker exists in the extracellular space. Inconsistent with this conclusion however, is the prior determination of a functional PKA phosphorylation site in the M3-M4 linker of GluR6. In order to test for the possibility of an intracellular region within the M3-M4 linker, we introduced a canonical PKA phosphorylation consensus sequence (RRASL) into the NR1 subunit. This mutation reduced the apparent affinity for glycine by more than two orders of magnitude without affecting the apparent affinity for glutamate. The IC50 for the competitive glycine antagonist, 5,7-dichlorokynurenate (DCK) measured at the EC50 for glycine was not affected. Individual point mutations at two component residues included within the introduced PKA consensus sequence (R_1 and L) each confer a reduction of glycine affinity over an order of magnitude. Taken together, the data suggest that we have localized residues that contribute to the glycine binding site. We have now mapped both extremes as well as a central region of the M3-M4 linker in the NMDA receptor NR1 subunit to the extracellular space using functional mutations.

781.5

KAINATE RECEPTOR SUBUNITS EXPRESSED IN SINGLE CULTURED HIPPOCAMPAL NEURONS: MOLECULAR AND FUNCTIONAL VARIANTS BY RNA EDITING. J. Rossier*, D. Ruano, B. Lambolez, A. V. Paternain and Juan Lerma. C.N.R.S. 91198, Gif-sur-Yvette, France. C.S.I.C. 28002, Madrid, Spain.

Five subunits, GluR5, GluR6, GluR7, KA-1 and KA-2, belonging to the ionotropic glutamate receptors of the kainate type have been cloned. Glutamate receptors that are activated by kainate, showing a rapidly desensitizing response, but not by AMPA, have been described in cultured hippocampal neurons. To determine the kainate receptor subunits which take part of native kainate receptors, we have applied a multiplex polymerase chain reaction of cDNAs reverse transcribed from mRNA harvested from single cultured hippocampal neurons after electrophysiological recording. We found that all the cells showing rapidly desensitizing currents in response to kainate express the GluR6 subunit mRNA and that some of them also express the GluR5 subunit mRNA. No GluR7, KA-1 or KA-2 subunit mRNAs were detected. Analysis of the editing sites of the GluR6 mRNA demonstrated that the three editing sites present in these subunits are edited to a different extent, and suggested that the Q/R site from the GluR6 subunit controls functional properties of native kainate receptors, similar to recombinantly expressed homomeric GluR6 receptors.

781.6

VISUALIZATION OF GLUTAMATE-GATED CHANNEL PERMEATION: HIGH RESOLUTION MAPPING OF NEURONAL SUBPOPULATIONS. R. E. Marc*, Moran Eye Center, University of Utah, Salt Lake City, UT 84132

Virtually all retinal neurons possess glutamate (Glu) receptors but the partitioning of AMPA/kainate (AMPA/KA) and NMDA subtypes is known for but a few of over 50 known cell types. Most Glu-gated cation channels are permeable to guanidinium$^+$ derivatives, including 4-aminoguanidobutane (AGB), and Glu-gated AGB accumulation can be visualized by anti-AGB IgGs. **Objective:** Map Glu-gated AGB permeation in all retinal neurons. **Methods:** Isolated goldfish retinas were challenged 15' *in vitro* with Glu agonists / antagonists and 25 mM AGB substituted for Na^+, then analyzed with multispectral protocols for deriving quantitative amino acid and AGB immunoreactivities. **Results:** All horizontal cell types, all identifiable OFF center bipolar cells (BCs), half of all GABAergic amacrine cells (ACs) and all but one glycinergic AC type demonstrated graded, CNQX-blocked AGB permeation gated by KA (5-125 µM) but no detectable signal after NMDA (3-3000 µM) exposure with or without Mg^{+2}. In particular, the unique cholinergic/GABAergic AC cohorts responded to AMPA/KA agonists alone. ON center BCs and solitary population of glycinergic ACs possess neither AMPA/kA- nor NMDA-gated responses. A large subset of GABAergic ACs displayed strong responses to both KA and NMDA.NMDA responses were blocked by AP-7 and MK-801. Most ganglion cells appear to bear both types of receptors although a small set seems driven exclusively by AMPA/KA systems. **Conclusions:** AGB permeation is a powerful tool for partitioning the Glu-gated responses of complex neuron populations. There are three forms of Glu-gated activation in retinal neurons: (1) Pure AMPA\KA systems; (2) Dual NMDA and AMPA/KA systems; (3) Those with neither. **Support:** NIH EY02576 and a Jules and Doris Stein Research to Prevent Blindness Professorship.

781.7

TRANSIENT AND PERSISTENT PHOSPHORYLATIONS OF AMPA-TYPE GLUTAMATE RECEPTOR SUBUNITS IN CEREBELLAR PURKINJE CELLS. K. Nakazawa[1*], S. Mikawa[2], T. Hashikawa[2], & M. Ito[1]. Lab. for [1]Synaptic Function and [2]Neural Systems, Frontier Res. Prgm., The Inst. of Phys. and Chem. Res.(RIKEN), Saitama 351-01, Japan.

Ionotropic glutamate receptor (GluR) phosphorylation is postulated to play a role in receptor desensitization and long-term synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD). To investigate the relationship of AMPA receptor phosphorylation with synaptic plasticity, we generated two kinds of antibodies against a phosphorylated peptide containing Ser-662 (that is a monoclonal antibody named **9PM**) or Ser-696 (a polyclonal antibody named **12P3**) of rat GluR2. Immunoblot analyses showed that both antibodies recognized subpopulations of cerebellar AMPA receptor subunits, which were phosphorylated. 12P3-immunocytochemistry revealed that a brief exposure of a rat cerebellar slice to AMPA leads to transient phosphorylation of the AMPA receptor subunits in Purkinje cell dendrites, providing evidence of activity-dependent receptor phosphorylation in a cell-type specific manner. An immuno-electronmicroscopic analysis showed that postsynaptic AMPA receptors at parallel fiber-Purkinje cell synapses were phosphorylated following AMPA application. Furthermore, persistent phosphorylation over 30 min was obtained when the exposure to AMPA was preceded by a 15 min perfusion of the slice with 8-bromo-cGMP, dibutyryl-cGMP or calyculin A, the stimuli causing long-term desensitization of Purkinje cell AMPA receptors. In contrast, marked 9PM-IR was observed in Bergmann glial cells regardless of stimuli causing LTD. These findings support the view that phosphorylation of GluR channels is of critical importance in the regulation of synaptic efficacy. Although our present results are inconsistent with three transmembrane model of GluR, our intracellular receptor phosphorylation might be explained if GluR membrane topology is different in a cell-type and/or subunit specific manner. Alternatively, it may be changed dynamically according to synaptic activity.

781.8

SINGLE-CHANNEL ANALYSIS OF A HIGH AFFINITY Ca^{++} AND Mg^{++} BINDING SITE IN THE PORE OF WILD TYPE AND MUTANT RECOMBINANT NMDA RECEPTORS. A. Auerbach* and L. Premkumar, Dept. Biophysics, SUNY at Buffalo, Buffalo NY 14214.

Residues at the 'QRN'site within the pore of glutamate receptors influence Ca^{++} permeability and Mg^{++} block. Mouse NMDAR ζ1 and ε2 subunits bearing either an N (wild type) or a Q at this position were expressed in oocytes and single-channel currents were recorded from outside-out patches. In the absence of Ca^{++}(1.5 mM EGTA) currents from receptors with a Q at the QRN site in both subunits (QQ) had both a main and a sublevel with conductances of 95 and 62 pS, respectively. NN receptors had a single main level with a conductance of 86 pS. These levels were differentially sensitive to Ca^{++}. The QQ sublevel conductance was reduced by 57 pS (Kd= 5 µM) and the QQ main level was reduced by 80 pS (Kd=205 µM). The NN main level conductance was biphasic with reductions in conductance of 17 pS (Kd=1.1 µM) and 32 pS (Kd=506 µM). For all forms of pore there was a residual conductance not blocked by Ca^{++} and carried by Na^+ (5,15, and 37 pS, respectively) suggesting that Na^+ can still permeate when Ca^{++} is bound. The QQ sub- and main levels were also differentially sensitive to Mg^{++}, with apparent Kds of 26 and 90 µM. Rate constants were obtained by analyses of QQ sublevel noise spectra and I/V curves. Ca^{++} binds to a site that is ~0.2 through the field (from the extracellular side) at a rate (-80 mV) of ~$4x10^9 M^{-1}s^{-1}$, unbinds at 4400 s^{-1}, and permeates at 17500 s^{-1}. Similar analyses indicate that Mg^{++} binds to a site that is ~0.28 through the field at ~$0.8x10^9 M^{-1}s^{-1}$, unbinds at 17500 s^{-1}, and permeates at 6200 s^{-1}. We conclude that the QRN site binds divalent cations with high affinity and at a high rate and defines a region near the mouth of the pore that does not require single file ion transport. Selectivity of Ca^{++} over Mg^{++} mainly arises from a higher permeation-to-unbinding ratio *(supported by NSF:IBN 9102232 BIR9403225).*

781.9

COEXPRESSION OF KA2 ALTERS THE PROPERTIES OF HOMOMERIC GLUR6 ION CHANNELS. J.R. Howe*. Department of Pharmacology, Yale University School of Medicine, New Haven, CT 065210-8066.

Patch-clamp techniques were used to compare the properties of homomeric and heterodimeric kainate-type ion channels. HEK 293 cells were stably transfected with the fully edited (VCR) version of the glutamate-receptor (GluR) subunit GluR6 or with GluR6 and the GluR subunit KA2. Steady-state currents were measured in cells pretreated with concanavalin A to remove desensitization.

As first shown by Herb *et al.* (Neuron 8: 775-785, 1992), cells co-transfected with cDNAs encoding the GluR6 and KA2 subunits responded to AMPA (0.2 to 1 mM), whereas cells transfected with GluR6 alone did not. Coexpression of KA2 also decreased the apparent affinity of kainate; the mean (±s.e.m.) EC_{50} values for kainate were 0.47 (±0.11) µM and 1.64 (±0.29) µM, respectively, in cells expressing GluR6 alone and cells co-transfected with GluR6 and KA2 (n = 7 and 4 cells). Hill coefficient (n_H) values also differed; the mean n_H value obtained from cells expressing GluR6 alone was 1.47 (±0.11) and the mean n_H value obtained from the GluR6/KA2 cells was 0.88 (±0.13). Agonist-evoked current noise was analyzed to estimate the unitary conductance (γnoise) of each type of recombinant channel. Spectral density analysis gave a mean γnoise value of 215 (±15) fS for cells expressing GluR6 alone (16 spectra from 14 cells), whereas the mean γnoise value obtained from GluR6/KA2 cells was 582 (±24) fS (16 spectra from 10 cells). The results suggest that homomeric GluR6 channels differ from heterodimeric channels formed from GluR6 and KA2 subunits both in their affinity for agonists and in their unitary conductance. (Supported by NS 30996).

781.10

EVANS BLUE SLOWS DESENSITIZATION AND INHIBITS GLUR6-MEDIATED CURRENTS IN TRANSFECTED HEK-293 CELLS. L. A. Raymond*. Dept. of Psych., U. of British Columbia, Vancouver, B.C.

The molecular cloning of several different glutamate receptor subunits has facilitated study of their structure, function, and modulation, and necessitated the development of subunit-specific antagonists in order to define the subunit composition of native neuronal receptors. Evans blue, a biphenyl derivative of 1,3-naphthalene disulfonic acid, was reported to selectively block kainate (KA)-evoked currents mediated by AMPA receptor subunits GluR1 and GluR2, but not by GluR3 or the KA-type subunit GluR6 (Keller et al., 1993). In that study in *Xenopus* oocytes, desensitization of GluR6-mediated currents was eliminated by treatment with the lectin concanavalin A (con A, 10 µM). In contrast to these results, we have found that Evans blue *does* block current through GluR6 receptors expressed in human embryonic kidney 293 (HEK-293) cells in the absence of con A. In GluR6-transfected cells, 1 µM Evans blue produced 91 ± 2 % (n=5) inhibition of the GLU or KA-gated current. Analysis of the remaining current revealed that desensitizaiton was slowed, with an increase in the decay constant (τ_D) from 7.8 ± 1.5 to 20.8 ± 4.8 msec (n=5). In agreement with the results of Keller et al. (1993), the inhibition of GluR6-mediated current by Evans blue was eliminated by pretreating the cells with 5 µM con A (which completely eliminated desensitization). Our results suggest that Evans blue may interact with more than one region of the ion channel (eg., ligand binding and desensitization domains). Furthermore, under the appropriate conditions (treatment with con A), Evans blue may prove a useful tool for differentiating neuronal AMPA and KA receptors.

781.11

EVIDENCE FOR MULTIPLE AMPA RECEPTOR COMPLEXES IN PYRAMIDAL NEURONS OF THE CA1/CA2 REGIONS OF THE HIPPOCAMPUS. R.J. Wenthold, A.S. Niedzielski*, J. Blahos and R.S. Petralia, Laboratory of Neurochemistry, NIDCD, NIH, Bethesda, MD 20892.

The four AMPA receptor subunits, GluR1-4, can form functional homomeric or heteromeric receptor complexes when expressed *in vitro*. Based on their physiological properties, it is thought that receptor complexes in neurons are heteromeric, comprised of two or more different subunits. While most neurons express multiple AMPA receptor subunits, it has not been determined if the complexes formed in any one neuron are of one type, or if the neuron is capable of forming multiple complexes which differ in their subunit compositions, and therefore, their functional properties. Hippocampal CA1/CA2 pyramidal neurons are an ideal model system for studying the composition of AMPA receptors in a neuronal population. These neurons are relatively homogenous in their functional properties and their abundant expression of GluR1, 2 &3, and a relatively pure preparation of the cell bodies and their dendrites can be obtained by dissection. Receptors were solubilized with Triton X-100. Western blots confirmed abundant GluR1, 2 &3 and low amounts of GluR4. Immunoprecipitation (IP) with anti-GluR2/3 antibodies, eliminated GluR2/3 staining on western blots, and reduced GluR1 staining to about 20% of control. To determine if this remaining GluR1 staining is due to functional receptors or disrupted or denatured complexes, ^{3}H-AMPA binding was done. IP with anti-GluR2/3 antibodies reduced binding to about 12% of controls; this could be reduced to about 3% by further IP with anti-GluR1 antibodies. This confirms the western blot data and suggests that about 10% of the ^{3}H-AMPA binding in this preparation is attributable to homomeric GluR1 receptors. IP of the solubilized preparation with anti-GluR1 antibodies eliminated GluR1 staining but reduced GluR2/3 staining only 50%. These results suggest two major populations of AMPA receptors are present in CA1/CA2 neurons, those made up of GluR1/GluR2 and those made up of GluR2 /GluR3. A population of homomeric GluR1 is also present.

781.12

DIFFERENTIATION OF GLIAL O-2A PROGENITORS REDUCES THE SENSITIVITY OF AMPA RECEPTORS TO BLOCK BY JORO SPIDER TOXIN Olimpia Meucci*, James A. Holzwarth, Alessandro Fatatis and Richard J. Miller Dept. of Pharmacol. and Physiol. Sciences, The University of Chicago, Chicago 60637 (IL).

We studied the properties of kainate-induced Ca^{2+} and Na^+ fluxes in cortical O-2A progenitor cells either before or after their differentiation into type II astrocytes. The experiments were performed using both O-2A primary cultures from rat cortex and the glial cell line CG-4 (which shares morphological and antigenic properties with O-2A progenitors and can differentiate in type II astrocytes or oligodendrocytes). Fura-2 based videoimaging and simultaneous monitoring of Ca^{2+} and Na^+ entry using microfluorimetry and whole-cell recording under voltage-clamp conditions were performed. The addition of kainate to undifferentiated or differentiated cells elicited inward Na^+ currents and large $[Ca^{2+}]i$ increases which were completely abolished by removal of extracellular Ca^{2+}, indicating the presence of Ca^{2+} permeable. Cyclothiazide, but not Con A, potentiated the effect of kainate or AMPA while GYKI 53655 prevented kainate-evoked Ca^{2+} and Na^+ fluxes, indicating the primary involvement of AMPA receptors. We examined the effect of Joro spider toxin (JSTx) and Argiotoxin 636, which only block AMPA receptors that lack GluR2(R). While these toxins were able to block kainate induced Na^+ currents and Ca^{2+} fluxes in the O-2A progenitors in a dose-dependent fashion, they were ineffective in differentiated cells. By testing the effect of JSTx during differentiation we observed a gradual increase of the number of cells insensitive to the toxin block which correlated perfectly with the expression of antigenic markers of differentiation. Thus, AMPA receptors in O-2A progenitors and type II astrocytes both flux Ca^{2+} efficiently but differ greatly in their sensitivity to spider toxins. This may indicate a change in the relative contribution of GluR2(R) subunits to individual AMPA receptors. (O.M. supported by ARIN)

781.13

INSULIN POTENTIATION OF NMDA RECEPTOR CURRENTS IN *Xenopus* OOCYTES: EFFECTS OF TYROSINE KINASES. S.J. Chen and J.P. Leonard* Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607.

Insulin receptors are present in mammalian brains as well as in *Xenopus* oocytes. As little as 80 nM insulin for 10 min potentiated the NMDA-activated currents on expression of heteromeric (ζ1/ϵ4) receptors in *Xenopus* oocytes. A 10X higher concentration of 0.8 μM insulin potentiated to 157% of control (157 ± 5% , n=37) reaching a maximum at 30 min. To test whether insulin potentiation was due to activation of tyrosine kinases, oocytes pre-treated for 30 min with 0.8 μM insulin were incubated in 100 μM genistein, an inhibitor of many tyrosine kinases. The insulin potentiation was reversed by genistein, but not by 100 μM daidzein, the inactive analog of genistein. We also injected exogenous tyrosine kinase pp60$^{c\text{-}src}$ into *Xenopus* oocytes expressing ζ1/ϵ4 receptors and found pp60$^{c\text{-}src}$ could potentiate NMDA receptor currents also (to 140 ± 7%, n=12). Both types of evidence indicated that tyrosine kinases were involved in potentiation of heteromeric NMDA receptor. Genistein also could inhibit the NMDA-activated currents in oocytes (to 81 ± 3% of control, n=4) in 30 min without insulin treatment. This suggests that there is basal tyrosine kinase activity in oocytes and that insulin stimulates this tyrosine kinase activity to potentiate NMDA receptor currents.

NEUROENDOCRINE REGULATION: OTHER III

782.1

SYNAPTOPHYSIN-IMMUNOREACTIVE INNERVATION IN THE RAT PITUITARY: MODULATION WITH AGING. L.C. Saland*, D. Ramirez and A. Apodaca, Dept. of Anatomy, Univ. of New Mexico Sch. Med.,Albuquerque, NM 87131

Synaptophysin (SN) is a glycoprotein found in vesicles of mature synapses, and is considered a marker for the presence of synaptic terminals. The pituitary gland of mammals is innervated by nerve fibers of hypothalamic origin which enter both the neural (NL) and intermediate (IL) lobes, with some studies showing anterior (AL) lobe innervation. Previous studies have shown reduced tyrosine hydroxylase and GAP-43 immunoreactive (IR) innervation in the aging pituitary. We examined the glands of younger and aged rats for SN-IR to determine if relative numbers of synaptic terminals are altered with aging. Adult male Sprague-Dawley rats, ages 8, 13 and 15-17 months were halothane anesthetized, perfused intracardially with buffered paraformaldehyde, and paraffin sections of pituitary tissue were immunostained for SN, using a monoclonal antibody (dilution 1:50) from Boehringer-Mannheim. Fine, punctate staining surrounded endocrine cells in the IL, with dense staining in the NL. No stained terminals were observed in the AL. The oldest rats exhibited a reduced staining pattern in the IL when compared to younger groups, suggestive of loss of mature synapses with advancing age. Supported by NIH NS21256, GM08139.

782.2

EVIDENCE FOR AXONAL SPROUTING IN THE ANTERIOR PITUITARY FOLLOWING ADRENALECTOMY IN THE RAT. G. Ju*, C.R. Lu, F.T. Meng, L.I. Benowitz. Department of Neuromorphology, Institute of Neurosciences, The Fourth Military Medical University, Xi'an, 710032 PR China and Laboratory for Neuroscience, Children's Hospital, Harvard Medical School, Boston, MA 02115, USA.

Our previous studies have demonstrated the presence of substantial amounts of peptidergic nerve fibers in the anterior pituitary of several mammalian species, including the rat. They were found to make synaptic contacts with the gland cells, indicating a direct neural regulation of this gland. Furthermore, the number of the calcitonin gene-related peptide-like immunoreactive (LI) nerve fibers in the anterior pituitary was found to increase significantly 4 days following adrenalectomy in the rat, implying an active response to changes of the hormonal levels of the animal. The present study aimed to investigate whether axonal sprouting could account for this increase. Twelve young adult male Sprague-Dawley rats (180 - 200g) were used. Six were used as normal control and 6 with bilateral adrenalectomy. Antibody against GAP-43 was used as the probe. In the normal controls a number of GAP-43-LI fascicles could be seen. Also present were individual varicose fibers. They were distributed mainly among the gland cells, although some could be found along the blood vessels. Four days following adrenalectomy there was a great enhancement of GAP-43 immunoreactivity in the anterior pituitary. Particularly striking was the dramatic increase in the immunoreactivity around the gland cells. Large numbers of GAP-43-LI varicosities of different sizes, many fairly thick, could be seen to gather around clusters of the gland cells, seemingly in close contact with them. The results imply an active axonal sprouting following this hormone manipulation and strongly support our hypothesis of neural-humoral dual regulation of the mammalian anterior pituitary.

782.3

POPULATION ANALYSIS OF SECRETORY RESPONSES FROM INDIVIDUAL RAT SOMATOTROPES DURING GHRP-6 POTENTIATED GH SECRETION. D.A. Leong, R.G. Smith, and M.D. Leibowitz*. Univ. Virginia, Charlottesville, VA 22908, and Merck Research Labs, Rahway, NJ 07065.

Hormone-stimulated secretory responses are intrinsically mediated by single cells. We have studied the population dynamics of individual cell responses under various conditions of growth hormone (GH) secretion. Using the quantitative reverse hemolytic plaque assay, GH release *in vitro* was measured from acutely isolated, single rat anterior pituitary cells over periods of 15-90 minutes. During this time, under basal conditions there was a cumulative increase in the number of plaques formed by GH secreting cells, without an increase in the area of individual plaques (suggesting that basal GH release is a stochastic process with only some cells active at any time). Either GHRP-6 or GRF produced dose-dependent increases in the number of secreting cells, again without detectable changes in magnitude. Failure to observe changes in the magnitude of individual responses strongly suggests that cell activation is followed by inactivation. At 15 minutes, maximal concentrations of GHRP-6 and GRF were not additive, while simultaneous application of both hormones at concentrations that produced little or no effect alone produced maximum secretion. Treatment with a cell-permeant analog of cAMP produced a dose-dependent increase in the number of GH plaques. The combination of maximal concentrations of GHRP-6 and cAMP was not additive, while submaximal concentrations of GHRP-6 and cAMP failed to potentiate the number of GH releasing cells, in contrast to the combination of GHRP-6 and GRF. Since GRF activates adenylyl cyclase this observation is striking. These data suggest that GHRP potentiates the number of GRF-induced cell responses at a step prior to cAMP production. Our findings indicate that GH release is a stochastic process at the level of individual cell responses and that hormones control the overall number of responding GH cells by acting at an early step in the intracellular signaling pathway.

782.4

GLUCOCORTICOID REGULATION OF PITUITARY GH-RELEASING HORMONE RECEPTOR EXPRESSION. O. Pinhas-Hamiel, P.J. Stevens, and P. Zeitler*. Div of Endocrinology, Child. Hosp. Res. Found. Cincinnati, OH 45229.

The effect of glucocorticoids (GC) on the GH secretory axis is complex. While GC is required for basal function of the axis, chronic GC excess *in vivo* inhibits GH secretion. The mechanism of this inhibition is unknown. Among other actions, GC excess results in blunting of the GH secretory response to GH-releasing hormone (GHRH), the primary hypothalamic regulator of GH synthesis and secretion. We tested the hypothesis that GC excess results in a down regulation of the pituitary receptor for GHRH. **METHODS**: Adult male Sprague-Dawley rats (n=6) were treated daily for 8 days with either the synthetic glucocorticoid dexamethasone (DEXA, 40μg/day) or vehicle. Pituitary GHRH receptor mRNA was quantitated by ribonuclease protection assay using an antisense ^{32}P-labeled cRNA probe specific for the 3'-coding region of rat GHRH receptor (a gift of K. Mayo, Northwestern Univ.). Pituitary GH mRNA was quantitated following northern hybridization. **RESULTS**: Chronic treatment of rats with DEXA resulted in 68% decrease in pituitary GHRH receptor mRNA (52±21 vs 17±8 AU/pit, p<0.01). In addition, DEXA treatment resulted in a 36% decrease in pituitary GH mRNA (26±4 vs 16±1, p<0.05). **CONCLUSIONS**: Chronic GC excess results in decreased pituitary GHRH receptor mRNA content. Decreased receptor synthesis may account, at least in part, for the observed blunting of response to GHRH. GHRH is an important physiologic regulator of GH gene expression *in vivo*. Although GC directly induces pituitary GH gene expression *in vitro*, the down-regulation of the GHRH receptor may account for the observed decrease in pituitary GH mRNA content.

782.5

CALCIUM SIGNALLING IN NORMAL LACTOTROPES. K.Gregerson* M.Y. Ho, J.P.Y. Kao. Dept. Pediatrics, UMAB, Baltimore, MD 21201.

Intracellular [Ca^{2+}] is an important regulator of prolactin release. However, it is not known how frequency and/or amplitude of $[Ca^{2+}]_i$ fluctuations encode signalling information in lactotropes. Population responses may obscure an underlying complexity in single cells. Using fura-2, we monitored $[Ca^{2+}]_i$ in single lactotropes isolated from proestrous female rats. As previously reported, unchallenged lactotropes fell into two functionally distinct groups: those with stable $[Ca^{2+}]_i$ which was not acutely sensitive to extracellular Ca^{2+}; and those with spontaneous fluctuations in $[Ca^{2+}]_i$ which were dependent upon influx of external Ca^{2+}. There was striking variability in the $[Ca^{2+}]_i$ patterns of the latter group, ranging from irregular, noisy fluctuations to rhythmic, repetitive oscillations with characteristic rise and decay kinetics. Quiescent cells could be activated to produce $[Ca^{2+}]_i$ fluctuations by the application *and withdrawal* of 100nM dopamine. Again, the character of the induced $[Ca^{2+}]_i$ activity showed tremendous cell-to-cell variation. In contrast, in any given cell, the pattern of $[Ca^{2+}]_i$ fluctuations was remarkably characteristic in reponse to repeated challenges. The interplay of multiple mechanisms (influx, buffering, sequestration, extrusion) determine $[Ca^{2+}]_i$ patterns and may be utilized differently among functionally diverse subpopulations of lactotropes. Modulation of Ca^{2+} handling could alter the relative proportion of lactotrope subpopulations and thereby be an important mechanism in the regulation of prolactin release in varying physiological states.

782.6

Cfos/*cjun* PROTOONCOGENES MEDIATE GLUCOCORTICOID STIMULATION OF TRH GENE EXPRESSION IN HYPOTHALAMIC NEURONS. L-G Luo* and I.M.D. Jackson Division of Endocrinology, Rhode Island Hospital, Brown University School of Medicine, Providence, RI 02903.

In fetal diencephalic cell cultures, we have shown that TRH mRNA is colocalized with the *cfos*/*cjun* mRNAs and that the expression of all 3 genes is enhanced following exposure to glucocorticoids(Luo and Jackson Prog 24th Ann. Meet. Soc. for Neurosci.,1994). In order to determine whether *cfos*/*cjun* mediated glucocorticoid stimulation of TRH gene expression, we studied the effect of antisense oligonucleotides directed against the first 18 bases of *cfos* and *cjun* mRNAs following the start codon sequence. To enhance stability phosphorothioate derivatived oligonucleotides were used. After preincubation for ten days, hypothalamic neurons in monolayer culture were exposed to 10 *um* antisense oligos of *cfos* and *cjun*, both individually and together, for 24 hours, after which dexamethasone (Dex 10^{-8}M) was added to the culture medium for an additional three days. The corresponding sense oligomers were used as controls. The cell TRH peptide content was assayed by RIA, and TRH mRNA was determined by *in situ* hybridization. The cell mean intensity was analyzed by an image analysis system and expressed in arbitrary units(au). Dex enhanced TRH peptide content 1.7 - 2.3 fold compared with control (262+/-16*vs* 153+/-8 fmol/well; p<0.01) and TRH mRNA levels 1.9 - 2.6 fold (31.2+/-2.1 *vs* 15.3+/-1.1 au; p<0.01). The addition of antisense *fos* or antisense *jun* individually did not affect the capacity of Dex to increase TRH peptide or mRNA levels. However, *cfos*/*cjun* antisense oligomers, when added together, completely prevented any Dex increase in the levels of either TRH peptide (124+/-7 fmol/well) or mRNA (14.89+/-0.82 au) *vs.* Dex treated group(p<0.01).*cfos*/*cjun* antisense oligomers did not alter the unstimulated TRH peptide level. These results are similar to our findings with TRH in anterior pituitary cells (Luo & Jackson, Program 77th Annual Meeting of The Endocrine Society, Washington DC., 1995). We conclude that *cfos* and *cjun* are involved in mediating glucocorticoid stimulation of TRH gene expression in fetal rat hypothalamic neurons.

782.7

COLOCALIZATION OF AROMATASE AND ANDROGEN RECEPTOR IN NEURONS OF THE BED NUCLEUS OF THE STRIA TERMINALIS. M.Sar*[1] and E.M. Wilson[2]. Departments of Cell Biology and Anatomy[1], Pediatrics[2], and Biochmistry and Biophysics,[2] School of Medicine, University of North Carolina, Chapel Hill, NC 27599.

The conversion of testosterone to estrogen occurs in specific areas of the brain through the action of enzyme aromatase. Aromatase activity is greater in males than in females and testosterone treatment increases the activity in castrated males. In the present study we investigated whether certain aromatase neurons in the brain contain androgen receptor (AR). Co-localization of aromatase and AR was determined by dual immunoperoxidase method using antipeptide antibody aromatase (N.Harada, Japan) and AR32. Adult male rats intact or castrated, treated with testosterone propionate were perfused with Zamboni's fixative. Brains were fixed and 10μm thick serial frozen sections of preoptic-septal region were processed for dual immunostaining. Sections were first stained with AR32 antibody using diaminobenzidine tetrahydrochloride followed by immunostaining with aromatase antibody using 4-chloro-1-naphthol. Nuclear localization of AR and cytoplasmic localization of aromatase in neurons were detected in several areas of the brain including the bed nucleus of the stria terminalis. Approximately forty to fifty percentage of neurons in the bed nucleus contained AR while 20%-25% of neurons contained aromatase. Thirty to thirty-five percentage of aromatase neurons in lateral part of the bed nucleus contained AR. AR containing neurons in the medial part of the bed nucleus and in the lateral septal nucleus did not show aromatase immunoreactivity. The results demonstrate that a sub- population of aromatase neurons in the bed nucleus of the stria terminalis contain AR and further sugest androgen action in aromatase neurons (Supported by NIH Grant NS 17479).

782.8

HOMOLOGOUS UP-REGULATION OF SOMATOSTATIN RECEPTOR SUBTYPE SSTR2 EXPRESSION IN RAT ARCUATE NUCLEUS *IN VIVO*. G.S. Tannenbaum*, J. Turner, F. Guo and A. Beaudet. Depts. of Pediatrics, Neurology & Neurosurgery, McGill University, Montreal H3H 1P3, Canada.

In vitro studies using various cell systems have provided conflicting results regarding homologous regulation of somatostatin (SRIF) receptors, and information on whether SRIF regulates the expression of its own receptors *in vivo* is lacking. In the present study we examined, by *in situ* hybridization, the effects of prolonged exposure to the SRIF analog, SMS 201-995, *in vivo*, on mRNA levels of two SRIF receptor subtypes, SSTR1 and SSTR2, in rat brain using ^{35}S-labeled antisense riboprobes (kindly provided by G.I. Bell). Labeling densities in four regions - the arcuate, ventromedial (VMN) and suprachiasmatic (SCN) nuclei of the hypothalamus, and in cerebral cortex (CTX), were quantified by computer-assisted microdensitometry (Biocom). Three hours after the iv administration of 50 μg SMS 201-995 to adult male rats (n=6), there was a 46% increase (P<0.001) in the labeling density of SSTR2 mRNA-expressing cells in the arcuate nucleus compared to normal saline-pretreated controls (n=6). SSTR2 mRNA signal density was also augmented in the three other regions examined: VMN: +35%; CTX: +27%; SCN: +7%. In contrast, no changes in SSTR1 mRNA-expressing cells were observed after SMS 201-995 treatment in any of the four regions measured, consistent with the subtype selectivity of this SRIF analog (i.e. high affinity for SSTR2 but little or no affinity for SSTR1). These results demonstrate, for the first time, that SRIF preexposure *in vivo* up-regulates the expression of a subtype of its own receptors, SSTR2, within the central nervous system. Such regulation may play an important role in a variety of neural functions, including the neuroendocrine regulation of growth hormone secretion by the arcuate nucleus.

782.9

MODULATION OF CORTICOTROPIN RELEASING HORMONE RECEPTOR IN CELL CULTURE WITH ANTISENSE ODN THOMAS SKUTELLA, CHRISTIAN BEHL, JOSEPH CHRISTOPHER PROBST, ULRICH RENNER, ROBERT NITSCH*, FLORIAN HOLSBOER, Max Planck Institute of Psychiatry, Clinical Institute, Munich and Humbolt University, Institute of Anatomy, Humbolt University/Clinic Charite, Berlin, Germany

Distribution of corticotropin releasing hormone receptors (CRHR) mRNA in the brain and pituitary supports a role of CRH as a regulator of neuroendocrine and behavioral adaption to stress. To further investigate the function of CRHR 1, which is mainly expressed in the posterior pituitary, we studied effects of different encapped phosphorothioate antisense oligodeoxynucleotides (ODNs) corresponding to CRHR 1 mRNA. Antisense, sense and scrambled ODNs were tested on CRH receptor binding, ACTH release and cell survival in rat posterior pituitary and ATT20 cell cultures. Studies on uptake and intracellular distribution of encapped phosphorothioate ODN using FITC-labeled antisense and sense labeled ODN revealed that within 2-4 hours ODNs were accumulated in the cytoplasma. As revealed by rt-PCR antisense ODN produced a dose- increase in CRHR mRNA after 48-72 hours of treatment, whereas the scrambled ODN had no effect on the mRNA levels compared to untreated controls. CRH receptor binding revealed a dose-dependent reduction of CRH receptor binding after 48-72 hours of antisense treatment, while no significant effects were observed with sense and scrambled ODN controls. Further CRH-stimulated ACTH secretion and cAMP were reduced after 48-72 hours of antisense ODN treatment.72 hours after a single antisense ODN treatment a dose-dependent counterregulative increase of ACTH secretion, CRH binding and CRHR mRNA was observed, while no effects were detectable with sense and scrambled ODNs. Cellular uptake and antisense effect could be potentiated by using transfection system. Cell survival tests revealed only weak toxic effects of ODN treatment in pituitary cell culture. The results provide functional and neurochemical evidence that CRH receptor antisense ODNs produce different effects in vitro depending on parameters such as concentration, time-window and mode of application.

782.10

ORIGIN OF CORTICOTROPIN-RELEASING HORMONE INNERVATION OF THE SHEEP ADRENAL DURING FETAL, NEONATAL AND ADULT LIFE. T.J. McDonald*P.W.Nathanielsz and C.Li. Laboratory for Pregnancy and Newborn Research, Cornell University, Ithaca, NY 14853 (HD 21350)

Corticotropin-releasing hormone (CRH) immunopositive fibers are found at the adrenal cortico-medullary interface of fetal sheep starting as early as 100 days of gestation (dG) and increasing with age to adulthood (*SGI abstract #O22, 1994*). Splanchnicotomy reduces cortisol, but not ACTH secretion in acutely hypotensive fetal sheep (*Endocrinology 127:2328*) and exogenous CRH is as effective as ACTH in eliciting adrenal cortisol in hypophysectomised calves (*J. Physiol. 447:489*). These findings suggest that, in addition to endocrine control of adrenal function via pituitary ACTH, sympathetic CRH may exert direct neural control on the adrenal. Therefore the present study examined sheep spinal cord for CRH-positive neurons projecting via splanchnic nerves to the adrenal. **METHODS:** Thoracic (T_1 -T_{13}) spinal cords of 7 fetal sheep (age range 110 dG to term; term ~ 150 dG), 2 newborn lambs and 2 pregnant ewes and splanchnic nerves from 1 fetus (130 dG) and 1 ewe were immunostained using a polyclonal rabbit anti-CRH antibody. One fetus (120 dG) was injected with Fluoro-gold (200 nl, 2.5% in saline) into the left adrenal medulla, necropsied at 134 dG and immunostained for Fluoro-gold and CRH coincidence. **RESULTS:** Intermediolateral (sympathetic) CRH-immunopositive cells were found in all spinal cords: the fewest were found in the 110 dG fetus; older animals exhibited varying numbers. The splanchnic nerves from both fetus and ewe contained many immunopositive CRH fibers. Thoracic spinal sympathetic Fluoro-gold immunopositive neurons double labelled with CRH were seen only on the injected side. **CONCLUSION:** CRH immunopositive cell bodies in the sheep sympathetic spinal cord that project to the adrenal medulla via the splanchnic nerves may consititute an important pathway for modulation of adrenal function, especially in situations such as chronic stress where ACTH concentrations in peripheral plasma return to baseline but cortisol concentrations remain elevated.

782.11

RESPECTIVE ROLES OF ANGIOTENSIN II AND ANGIOTENSIN III IN THE CENTRAL REGULATION OF VASOPRESSIN SECRETION. S. Zini[1], M.C. Fournié-Zaluski, E. Chauvel, B.P. Roques, P. Corvol[1] and C. Llorens-Cortès*[1]. [1]INSERM U36 and [2]U266, 75005, Paris, France.

In the brain, evidence suggests the existence of an independent renin-angiotensin system (RAS) including angiotensin receptors, precursors and enzymes required for the formation and degradation of angiotensins. Among the effector peptides of the RAS, angiotensin II (Ang II) and angiotensin III (Ang III) are able to increase blood pressure, water consumption and vasopressin release. These effects seem to be mediated by type-1 angiotensin receptors.

In attempt to delineate the respective roles of Ang II and Ang III on the regulation of vasopressin secretion, we have first identified *in vivo* the metabolic pathways of these two peptides, then subsequently studied vasopressin release using selective inhibitors of these enzymatic pathways. For this purpose, we have recently developed selective inhibitors of aminopeptidase A (APA, EC 3.4.11.7) and aminopeptidase N (APN, EC 3.4.11.2), two enzymes which might be involved into the metabolism of Ang II and Ang III, respectively. We then infused in the mouse by the intracerebroventricularly (icv) route, [^{3}H]Ang II in the presence or absence of EC33 (3-amino-4-thio-butyl sulfonate, the APA inhibitor) or EC27 (2-amino-pentan-1,5-dithiol, the APN inhibitor) and measured levels of [^{3}H]Ang II or [^{3}H]Ang III in the hypothalamus by HPLC. These experiments indicate that *in vivo* Ang II is cleaved by APA to generate Ang III which in turn is metabolized by APN. Next, we studied the effects of EC33 and EC27 on the release of vasopressin. Ang II or Ang III (3 ng) were infused i.c.v. in the presence or absence of inhibitors (30 µg) and plasmatic vasopressin levels were estimated by RIA.

Preliminary results show that the APA inhibitor completely blocks the Ang II-induced release of vasopressin while the APN inhibitor potentiates Ang III-induced release of vasopressin. These data suggest that Ang III is one of the main effectors of the brain RAS involved in the regulation of vasopressin secretion and that Ang II possibly exerts its effect through its conversion into Ang III.

782.12

SUCKLING STIMULUS ACTIVATES BRAINSTEM NEURONS PROJECTING TO THE ARCUATE NUCLEUS OF LACTATING RATS. H.-J. Wang*, G.E. Hoffman and M.S. Smith, Department of Neurobiology, University of Pittsburgh, Pittsburgh, PA 15261

Arcuate nucleus (ARC) neuronal function changes in the presence of the suckling stimulus. This study investigated the neural pathways that may relay the somatosensory input to the ARC during lactation. Lactating rats were anesthetized on day 2 postpartum (PP) and retrograde tracer Fluoro-gold (FAu) was iontophoresed from a glass electrode into unilateral ARC. The rats recovered and continued to nurse their 8-pup litters. To introduce an acute suckling stimulus, the lactating rats were separated from their pups for 48 hr beginning on day 8 PP. On day 10 PP, pups were reunited with the dam. Two to four hr after the onset of resuckling, mother rats were sacrificed and the brainstems were processed for immunocytochemistry (ICC). The parabrachial nucleus (PB) was heavily labeled by FAu. The A1/C1 and A2/C2 areas also had many FAu-labeled cells and the majority of them were tyrosine hydroxylase (TH)-positive. Fewer FAu neurons were found in central gray, A5, A6 and the raphe nucleus; only a few labeled neurons in A5, A6 expressed TH. Double-label ICC demonstrated that the suckling stimulus activated cFos expression in many FAu-traced cells in PB and A1/C1. Fewer cFos-positive FAu cells were found in other areas. Triple immunostaining showed that a few TH neurons in A1/C1 projecting to the ARC were activated by the suckling stimulus. These data indicated that the suckling stimulus may affect ARC neuronal function primarily via the activation of PB and A1/C1 areas; other brainstem regions may also participate in this process to a lesser extent. Supported by HD14643 and NS28730.

ION CHANNELS: CELL FUNCTION III

783.1

FLUORESCENCE IMAGING OF INTRACELLULAR SODIUM IN RAT HIPPOCAMPAL ASTROCYTES. C. R. Rose* and B. R. Ransom, Dept Neurology, Yale University, Sch Med, New Haven, CT 06510.

We determined the intracellular sodium concentration ($[Na^+]_i$) and mechanisms of its regulation in cultured rat hippocampal astrocytes using fluorescence imaging (345/385 nm) of the Na^+ indicator SBFI (sodium-binding benzofuran isophthalate). Calibrations were performed in live cells after each experiment. Baseline $[Na^+]_i$ was 14.6 ± 4.9 mM (n=270) in CO_2/HCO_3^--buffered saline (T=37°C), decreasing by 0.15 ± 0.09 mM/min (n=22) following removal of extracellular Na^+ ($[Na^+]_o$).

$[Na^+]_i$ transiently increased by 4.9 ± 3.5 mM (n=23) when changing from CO_2/HCO_3^--free to CO_2/HCO_3^--buffered saline, possibly due to activation of inwardly directed Na^+-HCO_3^--cotransport. Furosemide (1 mM) or bumetanide (50 µM) decreased $[Na^+]_i$ by 2 mM indicating a steady inward transport of Na^+ via the Na^+-K^+-2Cl^--cotransporter under control conditions. Inhibition of Na^+,K^+-ATPase by ouabain (1 mM) or removal of extracellular potassium ($[K^+]_o$) caused an increase in $[Na^+]_i$ by 2.5 ± 0.9 mM/min (n=24) and 4.2 ± 1.8 mM/min (n=44), respectively.

$[Na^+]_i$ decreased with increases in $[K^+]_o$ within the range seen in the CNS during neuronal activity. Following elevation of $[K^+]_o$ from 3 mM to 8 or 13 mM, $[Na^+]_i$ declined by 5.8 ± 2.2 and 7.1 ± 2.9 mM, respectively (n=22). Perfusion with saline containing 2 mM $[K^+]_o$ increased $[Na^+]_i$ by 3.0 ± 2.3 mM (n=23). Changing $[Na^+]_o$ by 20 mM, in contrast, resulted in changes in $[Na^+]_i$ of less than 3 mM.

These results show that baseline $[Na^+]_i$ of cultured astrocytes is strongly dependent on Na^+,K^+-ATPase activity, and influenced by Na^+-HCO_3^--cotransport and Na^+-K^+-2Cl^--cotransport. The high sensitivity of $[Na^+]_i$ to changes in $[K^+]_o$ suggests a prominent role of Na^+,K^+-ATPase in extracellular K^+ homeostasis.

This work was supported by a fellowship from the Deutsche Forschungsgemeinschaft to C. R. R. and by NIH grants to B. R. R.

783.2

INVOLVEMENT OF K^+ AND Cl^- CHANNELS IN MICROGLIA FUNCTION L. C. Schlichter*, B. Ballyk, G. Sakellaropoulos, P. S. Pennefather, and D. J. Phipps, Playfair Neuroscience Unit, The Toronto Hospital, Toronto Western Division, Toronto, Ontario, M5T 2S8.

In the present study, highly purified cultures of proliferating microglia were established from neopallia of newborn rats and classified into three populations. Within 3 weeks of isolation, "primary cultured" cells that had not been treated with CSF-1 containing media expressed: (1) an inwardly rectifying K^+ current (K_{ir}) that was inhibited by micromolar concentrations of Ba^{2+} in the bathing medium; (2) an outwardly rectifying K^+ current (K_v) with many similarities to the cloned K_v 1.3 channel of lymphocytes, including block by nanomolar concentrations of charybdotoxin (ChTX) and margatoxin (MgTX); and (3) an anion current that is similar to Cl^- channels in human T lymphocytes, including permeability to Cl^- and HCO_3^- and block by flufenamic acid, NPPB (4-nitro-2-(3-phenylpropylamino)-benzoic acid) and IAA-94 (6, 7-dichloro-2-cyclopentyl-2, 3-dihydro-2-methyl-1-oxo-1H-inden-5-yl (oxy) acetic acid). Type 1 cells were passaged and maintained in culture for up to 6 weeks in the presence of CSF-1 containing supernatants from an astrocyte cell line (LM 10-5). Type 2 cells arose spontaneously in these flasks as rapidly proliferating cells that could be maintained for many weeks without added CSF-1. Both Type 1 and 2 microglia expressed K_{ir} and Cl^- current, but not K_v current. Proliferation of Type 1 and 2 microglia was stimulated by CSF-1 containing supernatants and inhibited by Ba^{2+} (K_{ir} blocker) and the Cl^--channel blockers, flufenamic acid, NPPB and IAA-94. In contrast, the K_v blockers ChTX and MgTX had no effect. These results provide evidence that K_{ir} and Cl^- channels are necessary for microglia proliferation, whereas K_v 1.3 channels do not appear to be necessary in these cultured microglia. MgTX and NPPB affected the tyrosine phosphorylation levels of several proteins before and after CSF-1 stimulation. Supported by grants from the Medical Research Council of Canada to L.C.S. and P.S.P., and a Savoy Foundation fellowship to B.B.

783.3

COMPARTMENTATION OF INTRACELLULAR NA^+ AND CL IN AN ISOLATED NERVE CELL. C. Fåhraeus, J. Schouenborg* and W. Grampp. Dept. of Physiology and Biophysics, University of Lund, Sölvegatan 19, S-223 62 Lund, Sweden.

The compartmentation of intracellular Na^+ and Cl^- in the slowly adapting lobster stretch receptor neurone was studied using electrophysiological and mathematical techniques. Measurements were made of potential shifts that could be induced by current pulses (10-100 s, 1 - 16 nA) when the neurone was superfused with GABA. Since the input resistance under these conditions is practically nil the potential shifts could be translated to Cl^- shifts close to the membrane innerface. Also, measurements were made of the pump current following intracellular Na^+ accumulation due to repetitive impulse firing (0.5 -16 s, 40 Hz). These findings gave evidence for a Na^+ shift, similar in size to the Cl^- shift, close to the membrane innerface. The data suggest that Cl^- and Na^+, under dynamic conditions, are not evenly distributed within the cell. Rather, there will be concentration gradients of 15 mM or more from the submembrane region of the cell to the inner bulk of the cell. Further, the time course for the decay of the concentration gradient is approximately 5 - 25 seconds. The slow time course indicates that there must be a diffusion hindrance close to the membrane innerface. It is noted that a diffusion hindrance in the submembrane region of the cell is functionally advantageous in that it keeps concentration changes to the submembrane region. Thereby it also amplifies ion-activated processes (pumps, channels, transporters) associated with the plasma membrane.

783.4

ABNORMALITY IN THE ACTIVATION OF Ca-ACTIVATED POTASSIUM CHANNELS REVEALED IN STUDIES OF NEUROFIBROMIN-DEFICIENT PC12 CELLS. Heidi Scrable and Diane Lipscombe*, Depts. of Neuroscience, University of Virginia, Charlottesville, VA 22908 & Brown University, Providence, RI 02912.

Individuals with Neurofibromatosis-1 (NF1) manifest electrophysiological abnormalities in transmission of nerve impulses along both peripheral and auditory nerves. As a first step toward understanding the etiology of this abnormal electrical signalling, we have used whole cell and single channel recording to characterize the ionic currents expressed in neurofibromin(Nfb)- deficient PC12 cells. We report that Nfb-deficient PC12 cells have a significantly altered ionic profile compared to their wild-type counterpart. The most prominent difference from wild-type cells was the complete lack of an Iberiotoxin-sensitive maxi-K-type current in 7/7 whole cell recordings from Nfb-deficient PC12 cells. Concomitantly, there was no K_{Ca} channel activity in single-channel recordings from patches of Nfb-deficient PC12 cells in the cell-attached mode. However, the addition of μM Ca^{2+} to the cytosolic surface of excised patches induced the activation of high conductance K_{Ca} channels, suggesting that the channel is expressed but inactive. We detected an RNA species of ~4.5 kb that hybridized to a *slow-poke* cDNA probe on Northern blots of total RNA from both mutant and wild-type cells, confirming K_{Ca} expression. Thus, our results suggest an uncoupling, in Nfb-deficient cells, of maxi-K channels from the supply of calcium normally required for activation. We are now focusing our studies on voltage-gated calcium channels, which ordinarily provide the main route of calcium entry for activation of the maxi-K during excitation. Our preliminary studies indicate that voltage-gated calcium channels are also expressed in Nfb-deficient PC12 cells and that perhaps there is a deficit in the mechanisms which govern K_{Ca}/Ca channel co-localization. We are also testing the hypothesis that these changes, in Nfb-deficient PC12 cells, are a direct consequence of mutations in the *NF1* locus by comparing the ionic properties of neurons from *NF1* heterozygous and homozygous mutant and wildtype mice.

783.5

SINGLE CHANNEL PROPERTIES OF NEUROTENSIN-INDUCED CURRENT IN CULTURED VENTRAL TEGMENTAL AREA NEURONS. P.-Y. Chien[1*], R. H. Farkas[1], S. Nakajima[2] and Y. Nakajima[1]. Dept. of Anat. and Cell Biol.[1] and Dept. of Pharmacol.[2], Univ. of Illinois at Chicago, College of Med., Chicago, IL 60612.

Neurotensin (NT) excites dopaminergic neurons cultured from the ventral tegmental area primarily by inducing a non-selective cation current. This NT-induced inward current was blocked by the non-peptide NT antagonist SR48692 (Farkas, et al., Soc. Neurosci. Abstr. 19:1263, 1993). The NT-induced current was increased by decreasing the external Ca^{2+} concentration, suggesting block by external Ca^{2+} (Chien, et al., Soc. Neurosci. Abstr. 20:1528, 1994). Therefore, Ca^{2+}-free external solution was used for single channel studies. Single channel activity induced by NT could be recorded with the outside-out patch, indicating that the channel was activated locally. The mean inward single channel current was 2.1±0.06 pA (n=7); (holding potential = -80 mV; $[Na^+]_o$ = 155 mM; $[K^+]_o$ = 5 mM; $[K^+]_i$ = 153 mM). The mean channel open time was 0.28±0.02 ms. The closed time histogram was fit by two exponentials, suggesting that these channels opened in bursts. The NT antagonist SR48692 suppressed the channel activity by decreasing the channel open probability. Thus, the activity of the channel was mediated by the NT receptor. These results indicate that this channel underlies the NT-induced non-selective current recorded with the whole-cell configuration. Supported by NSF grant IBN 9319456.

783.6

MEMBRANE STIFFNESS AND CHANNEL FUNCTION: A GENERAL MECHANISM FOR MODULATING MEMBRANE PROTEIN FUNCTION? J.A. Lundbæk[1,2], P. Birn[2], A.J. Hansen[2], and O.S. Andersen*[1]. Cornell University Medical College USA[1], Novo Nordisk A/S, 2760 Måløv, Denmark[2].

The function of voltage-dependent calcium channels is modified by a variety of amphipatic compounds, but many of these compounds modify the function of other ion channels as well. This raises the question whether the alterations in calcium channel function reflect a more general mechanism involving altered interactions between the lipid membrane and the embedded proteins.

In experiments on gramicidin channels in planar bilayers and on N-type calcium channels using patch-clamp in IMR32 cells, we show that the synthetic agents Triton X-100 and β-octyl-glucoside, which decrease the stiffness of lipid membranes, reversibly *promote* calcium channel inactivation. Cholesterol which increases membrane stiffness, *inhibits* channel inactivation. The voltage activation of the calcium channels is not affected by any of the compounds. In the concentrations used none of the compounds changed the membrane leak currents.

Alterations in membrane stiffness may constitute a general mechanism for modulating the function of membrane proteins. Changes in membrane stiffness can be predicted from the molecular shape of the membrane active compounds. This may provide for insights into the pharmacological effects of membrane-active compounds, as well as for the general effects of cell membrane lipid composition on the function of membrane proteins.

783.7

ON THE USE OF GENETIC ALGORITHMS FOR PARAMETER OPTIMIZATION IN COMPARTMENTAL MODELS OF HIPPOCAMPAL NEURONS. R. M. Eichler West* and G. L. Wilcox, Graduate Program in Neuroscience and Minnesota Supercomputer Institute, University of Minnesota, Minneapolis, MN 55455.

Most neuronal modeling to date has used parameter settings for ionic conductances derived by trial-and-error procedures. It is unclear whether these settings represent tenuous, unique spaces or robust, reasonable approximations of the values present in real neurons. This study investigated the use of *Genetic Algorithms* (GA) as a robust, efficient and reliable method for determining appropriate parameter spaces in compartmental models of hippocampal neurons. The parameters examined represent the spatial distribution of voltage-gated and/or calcium-dependent channels using kinetic equations of Traub et al (J Neurophysiol. 1991). GA represent several advantages over previous methods of parameter optimization, including convergence within a finite time (as opposed to trial-and-error or random walk methods) and the avoidance of becoming trapped in local minima (as opposed to one-step or hill-climbing methods).

Implementation required the development of a *fitness measure*, which is a score assigned to a given set of parameters based on the degree of correspondence between the extracted properties of the numerically generated time series and the "correct", or "highly fit" experimental observations. A *fitness filter* mathematically extracts properties of the simulated time series and compares them to a large number of experimental observations. These observations include, but are not limited to: resting potential, frequency-intensity plots, burst frequency plots, calcium concentration in response to somatic or dendritic stimulation, action potential (AP) threshold, AP height, AP width, input resistance, average afterhyperpolarization (AHP) voltage, and alterations in the previous measures in response to the simulated presence of a given pharmacological agent. Thus, the fitness filter contains an implicitly embedded database of experimental observations.

The data generated by the GA was also analyzed for parameter sensitivity, i.e. the range of values for which a given parameter elicits fit behaviors was determined. The results of this sensitivity analysis are of particular interest to those who study adaptive modifications in neurons.

(Supported by grants of Cray C90 supercomputer time to GLW from the Minnesota Supercomputer Institute.)

783.8

NA^+ and CA^{2+} CHANNELS THROUGHOUT BASAL DENDRITES OF REGULAR SPIKING NEOCORTICAL PYRAMIDAL NEURONS: IMPLICATIONS OF BURSTING WITH EGTA. Paul A. Rhodes*, Rafael Yuste, and David W. Tank. Palm Beach, FL 33480 and AT&T Bell Labs, Murray Hill, NJ 07974.

It has been suggested that bursts in intrinsically bursting (IB) neocortical pyramids are driven by a Ca^{2+}-current generated depolarization of the dendrites, and that dendritic Na^+ currents must also be present in these neurons, so that somatic firing may invade the dendrites and activate dendritic Ca^{2+} channels (Rhodes and Gray 1994). Regular spiking (RS) pyramids under ordinary conditions emit only single spikes, but when Ca^{2+} chelators such as EGTA are injected, RS pyramids produce repetitive bursts (Friedman and Gutnick 1989). To explain the bursting of RS neurons with EGTA while still accounting for their single spike output under ordinary conditions, it is hypothesized that RS pyramids possess both Ca^{2+} and Na^+ currents in their basal dendrites (Yuste et al 1994; Benke and Angelides 1993), but that ordinarily their effect is masked by Ca^{2+}-gated K^+ currents. To assess the feasibility of this hypothesis, compartment model simulations of layer III and V RS pyramids were developed which 1) reproduced in detail the responses to current step characteristic of RS neurons (e.g. Mason and Larkman 1990) and which also 2) produced repetitive bursts when Ca^{2+} buffering was enhanced. The resulting models of RS pyramids satisfing these requirements included both Na^+ and Ca^{2+} channels in their dendrites, but at lower densities than in the dendrites of IB pyramids, along with fast, medium, and slow $[Ca^{2+}]$-gated K^+ currents (Schwindt et al 1988). These results suggest that since both IB and RS neurons burst under appropriate conditions, they both incorporate dendritic Ca^{2+} and Na^+ channels not only in the apical trunk (e.g. Amitai et al 1992), but also in apical oblique and basal dendrites, which are not probed by trunk impalements. Further, these results indicate the potential for processes that suppress dendritic Ca^{2+}-gated K^+ currents to shift the firing mode of neocortical regular spiking pyramidal neurons towards bursting.

783.9

MUTANT MICE FOR THE VOLTAGE-GATED POTASSIUM CHANNEL Kv3.1 ARE SMALLER AND DISPLAY A DEFICIT IN MOTOR SKILLS.

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More than a dozen different voltage-gated potassium (K^+) channel genes have been cloned from mammalian tissues, however the contribution of individual K^+ channel types to cellular excitability is not known. The K^+ channel Kv3.1 is predominantly expressed in granule cells of the cerebellum, and it is first detectable around postnatal day 7 during cerebellar development. Kv3.1 also forms the *l*-type K^+ channel in T lymphocytes. To study the role of Kv3.1, we generated a Kv3.1-deficient mouse using homologous recombination in embryonic stem cells. Heterozygous mice ($Kv3.1^{+/-}$) did not exhibit an obvious mutant phenotype. Homozygous mutant mice ($Kv3.1^{-/-}$) are viable and fertile. $Kv3.1^{-/-}$ males and females have significantly lower body weights than their control littermates. Preliminary studies indicate no apparent mutant phenotype in external morphology and in the gross anatomy of the brain, including the cerebellum. $Kv3.1^{+/-}$ and $Kv3.1^{-/-}$ animals demonstrate similar spontaneous locomotor activities in the open field test. In the Rotorod test for coordinated locomotion, however, $Kv3.1^{-/-}$ animals perform worse than their heterozygous littermates in agreement with the finding that Kv3.1 is highly expressed in cerebellar granule cells. To study the role of Kv3.1 channels in autoimmune disease, the $Kv3.1^{-}$ allele is introduced in two mouse strains with inherited autoimmune disease. These experiments may provide information about the pathogenesis of the disease. (Supported by NIH grant NS-28407 and by the Kent Waldrep National Paralysis Foundation to R.H.J.)

OPIOID RECEPTORS II

784.1

CHARACTERIZATION OF MULTIPLE OPIOID BINDING SITES WITH [^{3}H]BUPRENORPHINE IN RAT BRAIN MEMBRANES.

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Buprenorphine (Temgesic®) was radiolabelled with tritium at the positions 15 and 16. The purified end-product has 2.35 TBq/mmol (63 Ci/mmol) specific activity. The radioactive label was stable under acidic conditions, but not in the presence of strong basis. Binding characteristics of [^{3}H]buprenorphine was evaluated in particulate membrane fractions of rat brain. Specific ligand binding was of high affinity, saturable and naloxone-sensitive i.e. opioid in nature. The binding interaction displayed reversibility, and high degree of stereospecificity as measured by enantioselective opioid compounds. In kinetic experiments, equilibrium binding was achieved in 40 min incubation at room temperature, and dissociation of the receptor-ligand complex occurred readily when initiated by the addition of unlabelled opioid ligands. Homologous competition experiments reveal that [^{3}H]buprenorphine binds to an apparently single set of binding sites with a K_d value of 2.1 ± 0.4 nM. The maximal number of these sites was found to be 1.35 ± 0.27 pmol/mg protein. The binding parameters were not significantly changed in the presence of 100 mM sodium ion confirming the mixed agonist-antagonist properties of buprenorphine. In rat brain the ligand showed a relative preference for mu and kappa binding sites although opioid delta sites were found also to be labelled. The radioligand is capable of labelling kappa opioid receptors in frog brain membranes and it is a promising tool for studying ligand - receptor interaction in the opioid system.

784.2

μ-RECEPTOR ANTAGONIST BINDING OF NALOXONE BENZOYLHYDRAZONE TO STABLY EXPRESSED MOR-1 DISPLAYS G-PROTEIN DEPENDENCE.

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Naloxone and naloxone benzoylhydrazone (NalBzoH) are μ-opiate receptor antagonists *in vivo* and against agonist induced inhibition of adenylyl cyclase. In calf striatal and neuroblastoma cell membranes, NalBzoH binding differs from traditional G-protein receptor antagonists in that its binding to μ-receptors is highly sensitive to guanine nucleotides. Since the presence of other opioid receptors in these tissues makes it difficult to accurately assess the interaction of NalBzoH with the μ-receptor, we have examined ^{3}H-NalBzoH binding in CHO cells stably transfected with MOR-1. One selected cell line, MOR-A, has the expected profile of μ-receptor selective binding and inhibition of adenylyl cyclase. CHO cells transfected with vector alone have no opioid receptor binding or function. Naloxone and NalBzoH fully reverse the inhibition of adenylyl cyclase produced by morphine and the full agonist DAMGO while having no activity themselves, confirming their antagonist character. ^{3}H- NalBzoH binding in these cells reveals a sensitivity to guanine nucleotides similar to that observed in calf striatal or neuroblastoma cell membranes. Pertussis toxin (PTX), but not cholera toxin (CTX), affects ^{3}H-NalBzoH binding as well. The high affinity NalBzoH binding to μ-receptors is dependent upon an association with G-proteins, suggesting an unusual mechanism of antagonism and the possibility that NalBzoH is an inverse agonist.

784.3

INHIBITION OF L-TYPE Ca^{2+} CHANNELS AND ADENYLYL CYCLASE BY CLONED μ- AND δ-OPIOID RECEPTORS IN GH$_3$ CELLS.

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The coupling between endogenous opioid receptors and various effector systems has been studied extensively. The cloning of opioid receptor genes has facilitated research on the behavior of a single subtype of receptor. Although there is an increasing amount of data available on the binding characteristics and coupling of these receptors to adenylyl cyclase, their interaction with Ca^{2+} channels has not been tested.

To investigate the coupling of μ- and δ-opioid receptors to Ca^{2+} channels, we have stably expressed these receptor cDNAs in GH$_3$ cells. GH$_3$ cells express endogenous somatostatin (SRIF) receptors, voltage-gated Ca^{2+} and K^+ channels, but lack functional opioid receptors. Cells transfected with the μ-opioid receptor alone (GH$_3$MOR) bound both the non-selective opioid ligand diprenorphine (K_d = 0.33 nM, B_{max} = 0.39 pmoles/mg protein) and the μ-selective ligand DAMGO (K_i = 1.0 nM). DAMGO dose-dependently inhibited adenylyl cyclase activity in GH$_3$MOR cells (IC_{50} = 21.9 nM). Using the whole-cell patch-clamp technique with Ba^{2+} as the charge carrier, we have found that like SRIF, DAMGO (1 μM) also inhibits voltage-activated L-type Ca^{2+} channels (IC_{50} = 104.9 nM, INH_{max} = 26.5%). This action of DAMGO was attenuated by both naloxone and pretreatment with pertussis toxin. The δ-selective ligand DPDPE (1 μM) inhibited Ca^{2+} channel activity to a much lesser extent (3.5 ± 2.1%, n = 9). In addition to the μ-opioid receptor, GH$_3$MOR cells were transfected with the δ-opioid receptor cDNA (GH$_3$MORDOR). Expression of the δ-opioid receptor was confirmed by ligand binding studies. Like DAMGO, DPDPE also inhibited both adenylyl cyclase and Ca^{2+} channel activity. DPDPE (1 μM) reduced Ba^{2+} currents by 18.9 ± 1.1%, n = 12) in the cotransfected cells. We are investigating the mechanism(s) of action of μ- and δ-opioid receptors on Ca^{2+} channels expressed in GH$_3$MORDOR cells.

784.4

AGONIST-INDEPENDENT OPIOID RECEPTOR INHIBITION OF Ca^{2+} CHANNELS IN TRANSFECTED GH$_3$ CELLS.

T. G. Hales* and E. T. Piros. Departments of Anesthesiology and Psychiatry, UCLA, LA, CA 90025.

Agonist-independent activation of adenylyl cyclase has been observed in cells expressing cloned β2-adrenergic receptors. In addition, mutant α2C-adrenergic and m4 muscarinic receptors spontaneously inhibit adenylyl cyclase activity when expressed in non-excitable mammalian cell lines (Ren *et al.*, JBC 268:16483, 1993; Migeon and Nathanson, JBC 269:9767, 1994). Constitutive activity has not been reported for cloned opioid receptors and evidence for spontaneous coupling to ion channels is lacking. We investigated agonist independent opioid receptor coupling to native Ca^{2+} channels in GH$_3$ cells transfected with cloned μ– and δ–opioid receptors.

With Ba^{2+} as the charge carrier, the whole-cell patch-clamp technique was used to record voltage-activated Ca^{2+} channel activity from GH$_3$ cells stably transfected with both μ– and δ–opioid receptors (GH$_3$MORDOR). Ba^{2+} currents were inhibited by the μ– and δ–specific agonists DAMGO (1 μM) and DPDPE (1 μM). Inhibitions by these agonists were reversed by depolarizing prepulses (-20 to +60 mV, for 100 ms) and were blocked by intracellular GDP-β-S (2 mM). Without agonist, depolarizing prepulses caused an increase in the current evoked by a test pulse to 0 mV. This facilitation was not seen when GDP-β-S was included in the electrode solution, but was enhanced dramatically by intracellular GTP-γ-S (500 μM), suggesting that current enhancement was caused by a reversal of G-protein mediated Ca^{2+} channel inhibition. Facilitation was not observed with or without GTP-γ-S in untransfected GH$_3$ cells.

Our previous studies of opioid receptor coupling to Ca^{2+}-channels in GH$_3$MORDOR cells suggest that activation of μ- and δ- receptors causes inhibition of L-type channels via pertussis toxin sensitive G-proteins (see Piros *et al.*, this meeting). The data described here provides evidence for agonist-independent inhibition of Ca^{2+} channels by cloned μ- and/or δ-opioid receptors expressed in GH$_3$ cells. We are investigating whether the constitutive activity is a property of one or both of these receptor subtypes.

784.5

SPECIFIC *IN VIVO* BINDING OF [^{11}C]NALTRIBEN TO *DELTA* OPIOID RECEPTORS IN MOUSE BRAIN. C. D. Arnett*, M. Sajjad, E. Akgun, and P. S. Portoghese. PET Imaging Service, VA Medical Center, and University of Minnesota, Minneapolis, MN 55417.

Antinociception measures *in vivo* have demonstrated a distinction between two subclasses of *delta*-opioid receptors, termed $delta_1$ and $delta_2$. The $delta_2$-opioid receptor is selectively involved in the antinociception effected by release of endogenous opioids during cold water swim-stress and in the development of morphine dependence in mice. To investigate the two subtypes of *delta*-opioid receptors *in vivo* with the ultimate goal of selectively imaging the $delta_2$-opioid receptor in the living human brain, we labeled the nonpeptide $delta_2$-selective antagonist naltriben (NTB) with carbon-11 for use in PET imaging studies. Preliminary biodistribution studies of [^{11}C]NTB in mice demonstrated a brain uptake of 1.1 percent of the injected dose per gram (%ID/g) at 30 min after injection with a decline to 0.84 %ID/g at 60 min. Pretreatment 1.2 μmol/kg of NTB s.c. 30 min before [^{11}C]NTB reduced the brain retention of this radioligand by 65 percent at 60 min after injection. Pretreatment with 1.3 μmol/kg s.c. of the $delta_1$-selective antagonist benzylidenenaltrexone (BNTX) 30 min before [^{11}C]NTB also reduced brain retention of [^{11}C]NTB (by 45 percent) at 60 min after injection. At the doses used in this study, BNTX and NTB selectively inhibited antinociceptive activity of Leu- and Met-enkephalins administered i.c.v. or i.t., respectively, suggesting that the enkephalins mediate their antinociceptive effects through interaction at $delta_1$-opioid receptors in the brain and at $delta_2$-opioid receptors in the spinal cord (Takemori, A. E. and Portoghese, P. S. **Eur. J. Pharmacol. 242**: 145-150, 1993). The present results demonstrate specific binding of [^{11}C]NTB to mouse brain *delta*-opioid receptors. Inhibition of *in vivo* binding by pretreatment with a pharmacologically selective dose of BNTX is an interesting finding which will require further work to elucidate the respective roles of $delta_1$ and $delta_2$ opioid receptors.

784.6

CHARACTERIZATION AND APPLICATION OF A POLYCLONAL ANTISERA TO THE DELTA OPIOID RECEPTOR. T.J. Crook, A. Payne, F. Porreca and J. Lai*. Department of Pharmacology, University of Arizona, Tucson, Arizona, 85724.

The amino and carboxy terminal domains of the cloned opioid receptors show the greatest degree of sequence divergence. Fluorescent immunohistochemical analysis of NG108-15 cells with antibodies raised against the C-terminal of the delta opioid receptor (DOR) showed a granular staining in the cytoplasm near the plasma membrane, the nuclei were however devoid of staining. A 20-base sense sequence to from the DOR (nucleotides 7-26) was labeled at the 5-end with Texas red and HPLC-purified. The uptake of this oligonucleotide (ODN) into NG108-15 cells, maintained under serum-free conditions, was monitored over a 24hr period. Quantitative analysis showed that only 30-40% of the cells were labeled after 24hr, and the staining was found in both the nucleus and the cytoplasm. The uptake was both time and concentration dependent. Preicubation of the cells with either an antisense or a mismatch ODN (50μM/day) prior to the 24hr incubation with the Texas-red labeled ODN (50μM) did not significantly alter the percentage of labeled cells. When these cells were labeled with the anti-DOR antiserum a significant inverse correlation was observed between the level of the tagged ODN in a cell and the density of DOR staining after antisense pretreatment. Pretreatment with a mismatch ODN has no effect on DOR staining. We have also studied the anatomical distribution of DOR-like immunoreactivity (DOR-LI) in the brain. Mice were treated for seven days with antisense or mismatch ODN (12.5μg i.c.v.x2/day). Following treatment, mice were perfused with 4% paraformaldehyde and brains were harvested. Cryostat sections were incubated with the DOR antibody and visualized by a peroxidase reaction. High densities of DOR-LI were seen in the olfactory bulb, cortex and caudate putamen as well as a number of other sites. Administration of the antisense ODN produced a qualitative decrease in DOR-LI in many brain areas especially those around the ventricular system. Mismatch administration produced no difference in DOR-LI when compared with untreated controls.

784.7

DISTRIBUTION OF KAPPA OPIOID RECEPTOR IMMUNOREACTIVITY IN BASAL GANGLIA: A LIGHT AND ELECTRON MICROSCOPIC STUDY. J. F. McGinty*, C.K. Meshul[1], W.T. Bohler, T. A. Patterson[2] and C. Chavkin[2]. Dept. of Anatomy and Cell Biology East Carolina University School of Medicine, Greenville, NC 27858-4354, [1] V.A. Medical Center Portland, OR 97201 and [2]Dept. of Pharmacology, U. of Washington, Seattle, WA 98195.

Antisera raised against oligopeptides were used to map the presynaptic and postsynaptic locations of the kappa opioid receptor with immunocytochemistry at light and electron microscopic levels. Polyclonal, affinity purified antisera, raised in rabbits against the unique amino acid residues 371-380 (C-terminus-KT2) and 300-312 (N-terminus-KE4), were characterized as having a high titer by ELISA and as demonstrating specific recognition of the full length kappa receptor in rat brain membranes as shown by Western blot analysis. Immunoperoxidase staining in rat tissue perfused with 4% buffered paraformaldehyde revealed kappa receptor immunoreactivity (IR) in dense patches of the dorsomedial shell of nucleus accumbens and in cell bodies and fibers of the caudate-putamen, substantia nigra, and VTA. Prominent axonal immunostaining, more effectively demonstrated by the KE4 than the KT2 antiserum, was also present in the ventral pallidum, subthalamic and entopeduncular nuclei. Ultrastructural analysis revealed KT2 immunoreactivity in dendritic spines and in presynaptic terminals which made asymmetrical and symmetrical contacts with unlabeled dendrites in nucleus accumbens. The immunostaining was blocked by preadsorption with 30 μM kappa receptor peptide. This study demonstrates kappa receptor-IR in cells and fibers in the basal ganglia previously demonstrated to contain kappa receptor binding. Supported by DA 03982, DA04123 and Dept. Veteran Affairs.

784.8

KAPPA OPIOID RECEPTOR AGONIST BLOCKS POTASSIUM-STIMULATED INCREASE IN EXTRACELLULAR GLUTAMATE LEVELS IN RAT STRIATUM. S. M. Rawls*, C. T. Whitlow, W. H. Church[1] and J. F. McGinty. Dept. of Anatomy and Cell Biology and [1]Dept. of Chemistry, East Carolina University School of Medicine, Greenville, NC 27858-4354.

Kappa opioid receptor agonists decrease basal and stimulated extracellular dopamine levels in the striatum and decrease psychostimulant-induced behaviors. In addition, glutamate receptor antagonists block the development of psychostimulant-induced behavioral sensitization. Therefore, the purpose of this study was to determine whether kappa opioid receptor stimulation affects extracellular glutamate levels in the striatum as determined by *in vivo* microdialysis and HPLC coupled with electrochemical and diode array detection. Male Wistar rats were implanted with a guide cannula 4 days before insertion of a microdialysis probe into the dorsal striatum. After a 3 h. washout period, 30 min. baseline samples were collected for 2h. followed by 80 mM KCl for 30 min. to evoke glutamate release. In a second group, 100 μM of the selective kappa opiate agonist, U69,593, was perfused through the probe for 30 min. followed by U69,593 plus 80 mM KCl. Dialysates were collected for 90 min. post-treatment and each 30 min. sample was divided into an aliquot for dopamine and one for glutamate. Glutamate samples were derivatized using 2,3-naphthalene dialdehyde (NDA) before HPLC analysis. The KCl-stimulated increase in extracellular glutamate in the striatum was blocked by simultaneous perfusion with 100 μM U69,593. The kappa opioid receptor antagonist, norbinaltorphimine, will be used to test kappa receptor mediation of U69,593's effects. This study indicates that kappa opioid receptors regulate extracellular glutamate levels in rat striatum. Supported by DA 03982.

784.9

ONTOGENESIS OF δ-OPIOID RECEPTOR SUBTYPES IN RAT BRAIN AND THE STIMULATORY EFFECT OF WEANING: AUTORADIOGRAPHIC COMPETITION STUDIES. L Kitchen*, F. M. Leslie, A. Borsodi, G. Toth, P. Melchiorri & L. Negri. School of Biological Sciences, University of Surrey, Guildford, Surrey, GU2 5XH, UK & Department of Pharmacology, University of California, Irvine, CA 92717, USA

We have evidence from behavioural studies that the stimulus of weaning a mother from her rat pups at day 21 activates a subtype of the δ-opioid receptor. In addition, membrane binding and autoradiographic mapping shows that weaning activates a population of δ-receptors, primarily in the frontal-parietal cortex, recognised by [^{3}H]-deltorphin I (^{3}H-DELT I) but not by [^{3}H]- Ile^{5,6} deltorphin II (^{3}H-Ile DELT II). To determine if these sites represent receptors with distinct pharmacology we have carried out autoradiographic competition studies using Ile DELT II and [D-Ser^{2}Leu^{5}Thr^6]-enkephalin (DSLET) as displacing ligands vs. ^{3}H-DELT I and ^{3}H-Ile DELT II on sections from weaned and non-weaned 25 day old rats. Coronal sections (20μm) were cut at the level of the caudate and adjacent sections used for determination of binding with each radioligand. Sections were cut over a total distance of 460μm for competition by DSLET or Ile DELT II (0.1-300nM). Slides were pre-incubated for 30 min, and binding carried out at room temperature in 50mM Tris HCl (pH 7.4) for 90 min using 7nM of each radioligand with non-specific binding determined using naloxone (10μM). Washed and dried sections were apposed to Hyperfilm, developed after two weeks and autoradiograms quantified by video-based computerised densitometry. Preliminary quantitation (n=3) shows that DSLET and Ile DELT II compete equally effectively for δ-sites labelled with ^{3}H-DELT I or ^{3}H-Ile DELT II (IC_{50} 10-30nM) in caudate and frontal-parietal cortex. Further, there were no indications of marked differences in competition by the δ-ligands in weaned and non-weaned groups. The data suggest that the δ-receptors activated by weaning during development show a similar pharmacology with respect to competition by two δ-ligands which have been designated as subtype selective.

784.10

COCAINE-INDUCED UPREGULATION OF MU OPIOID RECEPTOR MESSENGER RNA IN NUCLEUS ACCUMBENS IS MEDIATED BY DOPAMINERGIC MECHANISMS. B.M.Cox, A.V. Azaryan, L.J. Grimm *, B.J. Clock. Department of Pharmacology, Uniformed Services University of Health Sciences, Bethesda, MD 20814

We have investigated the possibility that chronic cocaine treatment alters the levels of mRNA for mu and delta opioid receptors in brain regions rich in dopaminergic innervation. Male Sprague-Dawley rats were treated with saline or cocaine (50 mg/kg/day) for 3 days, delivered by osmotic minipump. Expression of mu and delta opioid receptor mRNA in olfactory bulb, n. accumbens and caudate putamen (caudal and rostral parts) was estimated using quantitative competitive polymerase chain reaction assays following reverse transcription. No changes in the levels of mRNA for delta opioid receptor were detected after exposure to cocaine in any of the brain regions examined. A significant increase in the level of mu opioid receptor (MOR) mRNA was detected in n. accumbens after 3 days cocaine treatment. In caudate-putamen and olfactory bulb no change in MOR mRNA was observed. In situ hybridization analysis also indicated elevated levels of MOR mRNA in n. accumbens after cocaine treatment for 3 days, with little change in other brain regions. Both SCH 23390 and eticlopride, selective antagonists of dopamine D1 and D2 receptors, respectively, block this cocaine-induced upregulation of MOR in n. accumbens.

We suggest that endogenous opioid systems in n. accumbens, the brain region specifically associated with the reinforcing properties of addictive drugs, are regulated by dopaminergic mechanisms and influenced by cocaine treatment. Opioid mechanisms may contribute to the behavioral actions of cocaine. *(Supported by a grant from the National Institute on Drug Abuse).*

784.11

SIGMA RECEPTOR REGULATION FOLLOWING CHRONIC ADMINISTRATION OF THE NOVEL SIGMA LIGAND BD1047 A.C. Zambon*, B.R. de Costa, D.D. Truong and R.R. Matsumoto. University of California Irvine, Dept. of Neurology, Irvine, CA 92717; NIDDK, Laboratory of Medicinal Chemistry, Bethesda, MD 20892.

The binding of several neuroleptics to sigma receptors has brought to question the possible involvement of these binding sites in various movement disorders. The novel sigma ligand BD1047 has been shown to have no effect on its own, but to dose dependently attenuate dystonic postures produced by the prototypic sigma ligands DTG and haloperidol, suggesting BD1047 acts as a sigma receptor antagonist. In order to further evaluate this possibility, rats were chronically treated through the lateral ventricle with BD1047 (in artificial CSF, 10 nmol/hr) and artifical CSF alone for 7 or 14 days via osmotic minipumps. Preliminary data shows that treatment for 7 days with BD1047 results in an up-regulation of sigma receptors in whole brain (increased Bmax, no change in Kd) over regular untreated and CSF alone treated rats. Receptor levels were notably higher in the cerebellum and myelencephalon, lower in the cortex and hippocampus, and unchanged in the striatum when compared to CSF treated rats, suggesting competing/dual influences of the known antagonist vs. cytotoxic effects of BD1047 following 7 days of treatment. However, after 14 days of exposure with BD1047, there was a marked decrease in sigma receptors in whole brain, suggesting that the cytotoxic effects of the compound may predominate after prolonged administration.

CELL DIFFERENTIATION AND MIGRATION VIII

785.1

DEVELOPMENT OF OLFACTORY NEURONS AND GNRH CONTAINING NEUROENDOCRINE CELLS IN THE ZEBRAFISH OLFACTORY ORGAN. K. E. Whitlock* & M. Westerfield, Institute of Neuroscience, University of Oregon, Eugene, Oregon 97403 USA.

We are interested in the process by which the olfactory placodes give rise not only to primary sensory neurons of the olfactory system, but also to neuroendocrine cells. Gonadotropin releasing hormone (GnRH) cells that migrate from the olfactory placodes to the central nervous system (CNS) are required for development of sexual maturity. Using antibodies against different forms of GnRH, we find two distinct populations of GnRH positive cells located in different positions in the CNS. One population is present between the olfactory organs and the post optic commissure, and the other population lies in the anterior commissure of the telencephalon. To investigate whether these cells originate in the olfactory placodes, we labeled olfactory placodes with DiI and followed cell migration in living embryos. DiI-labeled cells left the placodes and traveled to the locations where we observed GnRH positive cells. In addition, we ablated the olfactory placodes and showed that no GnRH positive cells migrate into the brain. These data suggest that the immunoreactive cells migrate from the olfactory placodes into the CNS.

To learn about the origins and migration of these GnRH positive cells, we transplanted olfactory placodes from labeled donor embryos to ectopic locations in unlabeled hosts. Preliminary evidence shows that cells migrate away from the transplanted placode. We are confirming the identity of these migrating cells and examining the details of their migration pathways.

Finally, we are beginning to investigate the lineage relationship between the olfactory neurons and the GnRH positive cells with the aim of understanding the developmental and evolutionary link between the olfactory and GnRH systems. This work was supported by MDA (KEW) and NIH HD22486 (MW).

785.2

TANGENTIAL MIGRATION IN THE PROLIFERATIVE ZONES OF THE DEVELOPING CEREBRAL CORTEX. N.A. O'Rourke* and S.K. McConnell Department of Biological Sciences, Stanford University, Stanford, CA 94305

Nonradial patterns of cell migration appear to disperse clonally related cells within the tangential domain of the developing cerebral cortex. Imaging studies in living cortical slices have revealed tangentially migrating cells within the intermediate zone. Analysis of the orientations of migrating cells labeled with ^{3}H-thymidine reveals even higher levels of tangential migration in the subventricular zone. In addition, within the ventricular zone the neuron-specific antibody TuJ1 stains a subset of cells with the bipolar morphology characteristic of migrating neurons in both mouse (Menezes and Luskin, J. Neurosci. 14:5399,1994) and ferret (O'Rourke et al, Devel. 121(7) 1995). Many TuJ1-stained cells are oriented parallel to the ventricular surface, suggestive of tangential migration. To ask whether cells migrate tangentially in the cortical proliferative zones, we made focal injections of DiI into the ventricular zone of living neonatal ferrets and examined their brains 24 hrs later. DiI-labeled, tangentially oriented cells with bipolar morphologies were found in both the ventricular and subventricular zones up to 500 μm from the injection sites. Cells spread tangentially along both the rostrocaudal and mediolateral planes. At least some of these cells were TuJ1-positive, supporting the notion that the TuJ1-stained cells observed previously are in fact migratory. After 24 hours, we found no evidence for the random tangential dispersion of rounded cells observed previously in the ventricular zone by Fishell et al (Nature 362:636,1992). In contrast, our evidence suggests that elongated cells migrate tangentially over long distances in linear trajectories within both the subventricular and ventricular zones. Supported by NIH MH51864.

785.3

GRANULE CELL DIFFERENTIATION IS DEPENDENT ON INTERACTIONS WITH OTHER CELLS IN THE DEVELOPING CEREBELLUM. J. Alder*, N. K. Cho, S. Temple, and M.E. Hatten, Laboratory of Developmental Neurobiology, Rockefeller University, 1230 York Ave. NY, NY 10021

To study the mechanism of granule cell fate specification in the developing cerebellum, we have isolated very early granule cell precursors from the rhombic lip where they originate. In order to determine if isolated rhombic lip cells are competent to differentiate into granule cells, we cultured purified E14 rhombic lip cells as aggregates. Although the rhombic lip cells were dividing in vitro, they failed to extend neurites or stain with the granule cell marker TAG-1. By contrast, E17 EGL cells, which have just completed their migration from the rhombic lip over the anlage to form the EGL but have not yet undergone massive proliferation, are already competent to extend neurites in vitro. Together these data may suggest that some interaction between the rhombic lip cells and another cell type in the embryonic cerebellum during this early wave of migration is important for rendering them competent to differentiate. To determine if these cells are competent to differentiate in vivo, we implanted E14 rhombic lip cells or E17 EGL cells into P6 EGL. Both cell populations were able to migrate and differentiate into granule cells, suggesting that the difference in their behavior in vitro can be rescued by the P6 environment in vivo. To determine which cell type might provide the signal which renders rhombic lip cells competent to differentiate, we have co-cultured labelled rhombic lip cells on monolayers of various cell types and assayed for neurite outgrowth. E17, P0, and P6 EGL cells can rescue neurite outgrowth of rhombic lip cells to a larger extent than cells from the whole E14 anlage whereas COS cells cannot induce neurite outgrowth at all. Interestingly, the large cell fraction of the E17 anlage which contains glial cells, Purkinje cells, and interneurons was also capable of rescuing rhombic lip cells. We are currently investigating the cellular source of the activity and whether the activity is a diffusible or membrane-bound factor.

785.4

GLIAL MEMBRANE PROTEINS IN THE PLASMALEMMAL JUNCTION BETWEEN MIGRATING NEURONS AND RADIAL GLIAL CELLS REGULATE CORTICAL NEURONAL MIGRATION. E. S. Anton*, R. Cameron and P. Rakic. Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06514

To study glial membrane proteins that may contribute to the process of neuronal migration in the developing brain, we have developed a polyclonal antiserum (D4) and a monoclonal antibody (15D7) that recognize membrane proteins present at the plasmalemmal junction between migrating neurons and their radial glial substrate (Cameron and Rakic, J. Neurosci., 14: 3139). Here we tested whether these junctional membrane proteins contribute to the regulation of neuronal migration in the cerebral cortex. E17-18 coronal sections of rat cerebrum were cultured on nitrocellulose or cell-tak coated coverslips. After 24 hours, the attached slices were lifted off, leaving behind 'imprints' of cerebral wall containing glial cells with migrating neurons attached to them. Changes in the migratory behavior of neurons in these imprint cultures were monitored before and after the addition of purified D4 or 15D7 antibodies. The rate of neuronal migration on glial substrates was significantly reduced in the presence of these antibodies to the junctional domains. Antibody exposure often led to withdrawal of leading processes, and in some instances, to detachment of neurons from their glial substrates. Exposure to control antibodies or rabbit immunoglobulins had no detectable effect on neuronal migration. These results suggest that the glial junctional membrane proteins recognized by these antibodies are crucial for the maintenance of normal neuronal migration. Dismantling of neuron-glial junctional complexes formed by these membrane proteins may underlie neuronal detachment from glial migratory substrates at appropriate positions in the developing cortical plate. (Supported by NS22807)

785.5

DEVELOPMENTAL REGULATION OF MAP2 VARIANTS DURING NEURONAL DIFFERENTIATION IN VITRO. S. A. Przyborski and M.A. Cambray-Deakin. SPON: Brain Research Association. Dept. Biomedical Science, University of Sheffield, Sheffield S10 2TN, UK.

Microtubule-associated protein 2 (MAP2) is known to promote the assembly and stabilization of microtubules and hence may be important in neuronal differentiation. MAP2 consists of high molecular weight (HMW) proteins MAP2a, MAP2b and a low molecular weight (LMW) isoform MAP2c. These proteins are produced from a single gene by alternative splicing. In this study, we describe the expression of the various MAP2 mRNA isotypes and protein isoforms during the development of rat cerebellar granule cell neurons over a 21 day period *in vitro*. *In situ* hybridization was used to detect MAP2 mRNA isotypes which corresponded to HMW- and LMW-MAP2 proteins. The distribution of MAP2 mRNAs in the P7 cerebellar cortex compared favourably with the different stages of granule neuron development *in situ*. During early stages of neuronal differentiation *in vitro*, high levels of MAP2c mRNA were observed which gradually decreased as development progressed. Throughout the period studied, MAP2ab mRNA concentrations remained low although a small transient rise was noted during the first 14 div. The profile of MAP2 protein variants showed further developmental regulation. The expression of the LMW-MAP2c isoform closely mirrored that of its mRNA whilst HMW-MAP2b protein concentrations rose during the first 10 div and were maintained in older cultures. HMW-MAP2a appeared after 4 div and gradually increased throughout the remainder of the study. Clearly, the outline of HMW-MAP2 protein did not relate to its encoding mRNA and such disparity may be due to the operation of different transcriptional and/or posttranslational mechanisms. Immunocytochemical analyses of MAP2 variants provided further information concerning their localization during neurite outgrowth. These results describe the developmental regulation of MAP2 mRNA and protein variants and show that the profile of their expression relates to the formation of processes during the differentiation of granule neurons *in vitro*.

785.6

MEF2C ANTISENSE OLIGONUCLEOTIDES INHIBIT DIFFERENTIATION OF HIPPOCAMPAL NEURONS IN CULTURE. Dimitri Krainc*, Adriana Ferreira, Maria Carles, Kenneth S. Kosik, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital, Centèr for Neurologic Diseases, Brigham and Women's Hospital, and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Myocyte enhancer factor 2C (MEF2C) belongs to a superfamily of transcription factors sharing a highly conserved DNA-binding domain, the MADS box. Several members of the MADS family have been shown to mediate lineage specification and differentiation. Previously, we have shown that MEF2C is induced during neuronal differentiation and activates transcription in a site dependent manner (Leifer et al., *PNAS* 1993). Here, we used cultured rat hippocampal neurons to study the role of MEF2C in neuronal differentiation and neurite outgrowth. The addition of MEF2C antisense oligonucleotides (ODNs, 50 μM) reduced MEF2C levels, as determined by immunocytochemical analysis, and almost completely prevented neurite outgrowth. Similar concentrations of sense ODNs did not affect neurite elongation. In addition, the removal of antisense ODNs from the culture medium led to a nearly complete recovery of neurite outgrowth.

These results suggest that MEF2C might play a role in the transcription of neurospecific genes involved in neuronal differentiation and neurite elongation.

785.7

THE HEAT SHOCK RESPONSE OF PC12 CELLS IS DIMINISHED UPON NEURONAL DIFFERENTIATION: RELATIONSHIP TO KEY TRANSCRIPTION FACTORS.
D. S. Dwyer*, Y. Liu, S. Miao and R. J. Bradley. Departments of Psychiatry and Pharmacology, LSU Medical Center-Shreveport, Shreveport, LA 71130.

The production of heat shock proteins (Hsps), including Hsp70, Hsp60 and Hsp90, by PC12 cells was monitored as a function of neuronal differentiation. Normal, undifferentiated PC12 cells dramatically increased the synthesis of Hsp70 and Hsp60 upon heating or exposure to ethanol. A 30 minute exposure to 42°C or 24 hours of incubation in 0.3% ethanol was sufficient to significantly elevate the levels of these inducible Hsps. In contrast, PC12 cells that were induced to differentiate with nerve growth factor (NGF) failed to produce Hsp70 or Hsp60 in response to heat or ethanol treatment. Disappearance of the heat shock response of the cells was directly relate to the extent of neuronal differentiation (as judged by neurite outgrowth and cessation of division). Cellular levels of the constitutive proteins Hsc70 and Hsp90 were unaffected by differentiation. Production of Hsps was restored in the differentiated cells by removal of NGF which coincided with loss of the neuronal phenotype. To some extent, introduction of fresh serum also rekindled the cellular stress response. Analysis of the transcriptional control of Hsp production revealed a relationship between the levels of Hsps and key transcription factors based on the differentiation state of the cells. Attenuation of the heat shock response in terminally differentiated neuronal cells may explain the increased susceptibility of neurons to the harmful effects of environmental stress.

785.8

NEUROTROPHIC ACTIVITY OF STEM CELL FACTOR AND MELANOTROPHIC ACTIVITY OF NEUROTROPHINS IN NEURAL CREST CELLS. M. Sieber-Blum* and J. Saskowski. Dept. of Cell Biology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

The effect of relevant growth factors on neural crest cell development was determined by colony assay of quail neural crest cell proliferation and differentiation. Pluripotent neural crest cells are not only found in the migratory neural crest, but also at sites of terminal differentiation, including the dorsal root ganglion, the sympathetic ganglion and the developing skin. This indicates that signals from the embryonic microenvironment, such as growth factors, participate in the control of proliferation, survival and cell type specification. In the presence of stem cell factor (SCF), the following parameters were changed compared to control: 1) More colonies contained stage specific embryonic antigen-1 (SSEA-1) - immunoreactive sensory neuron precursors. 2) Colonies founded by pluripotent neural crest cells contained more cells due to a trophic influence of SCF as determined by bromodeoxyuridine incorporation studies. However, no effect of SCF on pigment cells was observed. By contrast, when SCF and one of the three neurotrophins tested (nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3) were added together to the culture medium, the size of fully pigmented colonies, which are founded by committed melanogenic cells, increased. Again, this was determined to be a trophic, not a mitogenic, influence by the SCF/neurotrophin combination. SCF was required by the cells from the beginning of the culture period, whereas the neurotrophin could be added significantly later, suggesting that in the presence of SCF melanogenic cells become neurotrophin-dependent. Taken together, the data indicate that SCF can have neurotrophic activity in the sensory neuron lineage and that the well-documented trophic action of SCF on pigment cells requires the presence of a neurotrophin. Supported by USPHS Grant HD21423 and a Research Grant from the Dysautonomia Foundation.

785.9

DYNAMIC ANALYSIS OF TRUNK NEURAL CREST MIGRATION IN THE AVIAN EMBRYO. C. E. Krull[1]*, A. Collazo[2], S. E. Fraser[2], M. Bronner-Fraser[1]. [1]Dev. Biol. Ctr., UC-Irvine, Irvine, CA 92717; [2]Dept. of Biol., Cal. Inst. of Tech., Pasadena, CA 91125.

Trunk neural crest cells migrate through the somites in a striking segmental fashion, entering the rostral but not caudal somitic sclerotome. Various attractive and inhibitory molecules are thought to influence the patterning of trunk neural crest cells. It has been difficult, however, to observe the dynamic aspects of neural crest migration *in vivo* and perturb candidate guiding molecules due to the lack of an accessible bioassay. To this end, we have developed a novel explant system. Trunk regions of the chick embryo, placed in explant culture, continue to develop apparently normally for two days; the morphological and molecular properties of the somites are comparable to those observed in intact embryos. Neural crest cells, recognized by prelabeling with DiI or by HNK-1 immunoreactivity, migrate in the somites of the explants in their typical segmental pattern. This system allows us to follow trunk neural crest migration in situ for the first time using time-lapse videomicroscopy. Neural crest cells frequently migrated in close-knit groups of 2-4 cells, with mean migration rates of 10-14 μm/hour. The migratory trajectories of individual neural crest cells were often complex, with cells migrating in an episodic manner. Treatment of trunk explants with the lectin peanut agglutinin (PNA) altered the patterning of neural crest migration and slowed the migratory rate. Cells migrated in both the rostral and caudal halves of the sclerotome with mean rates of migration ranging from 6-13 μm/hour. These results suggest that PNA-binding molecules are required for the segmental patterning of trunk neural crest migration. Experiments to examine the potential roles that other molecules may play are in progress. *Supported by NRSA/NINDS 09459 to CEK and USPHS HD-15527 to MB-F.*

785.10

GAP JUNCTION DOWN-REGULATION IS REQUIRED FOR NEURONAL DIFFERENTIATION. R. Rozental*, M. Urban, G.I. Fishman, F.-C. Chiu, E.M. Eves, M.R. Rosner, J.A. Kessler and D.C. Spray. Depts. of Neurosci. and Neurol., A. Einstein Coll. Med., Bronx, NY 10461; Inst. Biophysics, Fed. Univ. Rio Janeiro, Brazil; Ben May Inst., U. Chicago, IL 60637.

We recently showed that electrotonic coupling is quite strong among embryonic cells destined to become neurons, and that strength of coupling changes during neuronal differentiation (*Dev. Biol.*, 167: 350-362, 1995). However, it remained unclear whether the decline in neuronal coupling is an obligatory step in this process. To address this issue, we used an SV40 Tts-immortalized rat hippocampal neuronal cell line overexpressing bcl-2 (H19-7bcl2-R10s) that can be induced to differentiate at the non-permissive temperature (39°C) in defined medium containing basic FGF (bFGF) and retinoic acid (RA). We compared the immunocytochemical and functional characteristics of cells kept at 33°C or 39°C and with cells kept at 39°C and treated with bFGF (10 ng/ml) and RA (0.3 μM); these were compared with cells transfected with Cx43 [electroporation with 20 μg DNA: 4 μg of fluorescent protein expression vector (CMV-GFP), 16 μg of either Cx43 expression construct (pGF1) or a neomycin phosphototransferase-βgalactosidase fusion protein control construct (CMV-βgal-neo)]. GFP expressing cells were identified by epifluorescence. At 33°C, both parental and transfected cells are highly coupled by Cx43 and are not excitable. However, at 39°C and with RA and bFGF, parental cells progressively uncouple, as they express neurofilament protein (NFM) and membrane excitability. Constitutive expression of Cx43 in transfectants prevents the expression of neuronal phenotype. Thus, cellular uncoupling appears to be a necessary first step for cellular differentiation early during neuronal ontogeny.

785.11

MN20/D2 CYCLIN EXPRESSION REGULATES DIFFERENTIATION OF CEREBELLAR CELLS IN CULTURE CJ MacNabb* & ME Ross, Dept of Neurology, Univ of Minnesota, Minneapolis, MN 55455.

The D type cyclins are key regulators of the G1 to S phase transition of the cell cycle, and are an integral part of cell growth and differentiation. We previously cloned from cerebellum a message form, MN20, of the D2 cyclin gene(J. Neurosci, 14:6384, '94). MN20 has a highly restricted temporal and anatomic expression pattern in brain suggesting a role in the transition of the neuroblast from proliferation to a more mature neuronal morphology. In this study, we used antisense oligodeoxynucleotides (oligos) to investigate the effect of downregulation of MN20/D2 cyclin expression on the differentiation of cerebellar granule precursors in culture. Phosphorothioate derivatized, antisense (AS)oligos complementary to sequences in the 5' end of the cDNA and their sense controls were synthesized. Cerebellar granule precursor cells were isolated from mice on postnatal day 3-5, and plated in serum-free medium. After several hours, triplicate cultures were treated with 5, 10, or 20 μM AS-oligos and morphology of the cells was examined at 10-12 hr intervals. An AS1-oligo, targeted near the translation start codon, delayed neurite outgrowth up to 42 hr, while cultures receiving no or sense-strand oligos put out neurites by 24 hr after plating. The number of cells affected by this delay increased in a dose-dependent fashion. Many of the differentiation-delayed cells died in culture by 36 hr. Surviving cells extended poorly formed neurites starting around 42 hr, as determined by immunohistochemical labeling of neurofilament. The AS1-oligo effect on cell differentiation and survival was partially rescued by bFGF. The AS1-oligo effect was sequence specific since an oligo that hybridized to a region upstream of the AUG, and therefore was not expected to interfere with translation of the cyclin mRNA, had little effect on cultures. These results suggest that proper regulation of D2 cyclin expression is required for successful differentiation and survival of cerebellar granule neurons.

785.12

THE REGULATORY PROTEINS p53 AND BAX ARE INVOLVED IN NEURONAL AND OLIGODENDROCYTE CELL CYCLE REGULATION. O. Eizenberg[1]*, E. Gottlieb[2], A. Faber-Elman[1], M. Oren[2], V. Rotter[3] and M. Schwartz[1]. Depts. of [1]Neurobiology, [2]Chemical Immunology and [3]Cell Biology, Weizmann Institute of Science, Rehovot, Israel.

This study shows that the p53 tumor suppressor gene is involved in the regulation of differentiation and programmed cell death of neurons and oligodendrocytes, two cell types which leave the mitotic cycle very early in development and undergo massive cell death upon maturation of the nervous system. Evidence is provided that in this cascade of events another regulatory protein, bax, is also involved. Specifically, we show that primary rat oligodendrocytes and neurons, as well as pheochromocytoma cells, constitutively express the p53 protein. At critical points in the development of these cells, p53 undergoes a change in its subcellular localization: it translocates from the cytoplasm to the nucleus during differentiation, while in fully mature differentiated cells it appears again in the cytoplasm. These subcellular changes in localization were found to occur concomitantly with changes in the levels of both p53 mRNA and protein. The bax, known to be up-regulated by p53, was found to be expressed constitutively in the nucleus of these cells. p53 is also shown to play a role in the apoptotic death of such cells; infection with a recombinant retrovirus encoding a C-terminal p53 miniprotein, previously shown to act as a dominant negative inhibitor of endogenous wild-type p53 activity, was found to protect neurons and oligodendrocytes from spontaneous apoptotic death. We propose that p53 is recruited into the nucleus upon receiving appropriate signals and plays a regulatory role in directing neural cells toward either differentiation or apoptosis, probably by interaction in the nucleus with bax.

785.13

The role of p35/cdk5 kinase in neuronal differentiation and neurite outgrowth

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To date all known cyclin dependent kinases (cdks) have been linked to cell cycle progression, with the exception of one - cdk5. We have previously shown that this kinase is activated when complexed to its regulatory partner, p35. The expression pattern of p35 and thus the p35/cdk5 kinase activity are exclusive to post-mitotic neurons of the developing central nervous system. The first signs of p35 expression are during the initial stages of cell migration from the ventricular zone. Subcellular localisation of both proteins is cytoplasmic; p35 is highly expressed in neuronal growth cones.

To address the role of the p35/cdk5 kinase in neuronal differentiation and neurite outgrowth we have altered levels of this kinase in rodent primary cortical cultures by means of transfection, a method established by Hank Dudek in Michael Greenberg's laboratory. P35, cdk5 or a dominant negative cdk5 mutant (dncdk5) were ectopically expressed in E17 rat embryonic cultures from the strong Cytomegalovirus (CMV) early promoter. Transfected cells were distinguished by co-transfection with a CMV-β galactosidase or retroviral LTR-alkaline phosphatase vector.

We were able to cause a dramatic reduction of neurite length in cells over-expressing dncdk5, a phenotype rescuable by co-transfection with wild type p35 or cdk5. In contrast, cells over-expressing p35 and cdk5 had increased neurite lengths in comparison to mock transfectants. The results of our experiments therefore strongly suggest a direct involvement of the p35/cdk5 kinase in neurite outgrowth.

To examine the role of p35 and cdk5 in earlier stages of differentiation, we will use proliferating cortical precursor cells. The expression of several neuronal markers will indicate their stage of differentiation.

NEUROTOXICITY II

786.1

ACUTE NEUROTOXICITY OF 2'-NH_2-MPTP: CHANGES IN REGIONAL SEROTONIN, 5-HIAA, NOREPINEPHRINE, DOPAMINE AND GFA LEVELS IN SWISS WEBSTER MICE. A. M. Andrews*, D. B. Miller, J. P. O'Callaghan and D. L. Murphy, Laboratory of Clinical Science, NIMH, Bethesda, MD 20892-1264 and Health Effects Research Laboratory, US EPA, RTP, NC 27711.

Our previous work has shown that 2'-NH_2-MPTP induces substantial depletions in cortical and hippocampal serotonin and norepinephrine lasting as long as 6 months without concomitant decreases in striatal dopamine (Andrews, A. M. and Murphy, D. L., *J. Pharmacol. Exp. Ther.* **267**, 1432-1439, 1993). In the present study, we sought to extend our observations to include the acute effects of 2'-NH_2-MPTP on regional brain neurochemistry. Administration of 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2'-NH_2-MPTP; 4 x 20 mg/kg ip, at 2 h intervals) to mice caused large decreases in cortical and hippocampal 5-HT and NE measured 30 m, 2, 24, 48, and 72 h, and 7 and 21 days post-treatment. 5-HT was decreased by 70-90% and NE by 80-100% ($p<0.001$) at all times examined. Both 5-HT and NE were at their lowest levels within 30 m of the last injection of 2'-NH_2-MPTP. In contrast, the serotonin metabolite 5-HIAA was decreased only 20% at the earliest time-points in frontal cortex and hippocampus. 5-HIAA levels continued to fall until they were depleted ~60% at 48-72 h after treatment. Acutely, striatal dopamine was significantly decreased by 20-40% up to 48 h after the last injection of 2'-NH_2-MPTP but returned to control level by 72 h post-treatment where it remained throughout the 3 week period studied.

Astrogliosis was determined by immunoassay of time-dependent changes in glial fibrillary acidic protein in various brain regions. GFA levels were increased to 130% of control in cortex, hippocampus, and brain stem 48-72 h post-treatment ($p<0.05$); however, the observed increases following 2'-NH_2-MPTP were modest in comparison to previously reported increases on the order of 350% seen in striatal GFA at comparable times following MPTP treatment (O'Callaghan, J. P., Miller, D. B., and Reinhard, J. J., *Brain Res* **521**, 73-80, 1990).

786.2

INCREASED MANGANESE (Mn) CONCENTRATIONS AND CONCOMITANT LOSS OF POSTSYNAPTIC DOPAMINE D_2 RECEPTOR DENSITY IN PALLIDUM IN HUMAN HEPATIC ENCEPHALOPATHY (HE). R.F. Butterworth*, L. Spahr, D.D. Mousseau, G. Therrien and G. Pomier Layrargues. Liver Unit and Neuroscience Research Unit, Hopital Saint Luc, University of Montreal, Montreal, Quebec, Canada, H2X 3J4.

Pallidal lesions characterized by increased signal intensity on T_1-weighted Magnetic Resonance Imaging (MRI) have consistently been observed in cirrhotic patients with mild to severe HE. Since one potential cause of T_1 shortening is Mn deposition in brain, Mn concentrations were measured by atomic absorption spectrometry in autopsied brain tissue from 9 cirrhotic patients who died in hepatic coma and an equal number of control subjects. Mn concentrations were selectively elevated 3-fold in pallidum of HE patients (controls: 1.90 ± 0.40; HE patients: 5.40 ± 2.00 μg/g dry weight, $p<0.01$). In view of reports that chronic Mn toxicity results in alterations of the dopaminergic system, dopaminergic D_1 and D_2 receptor densities were measured by in vitro radioligand binding techniques in the same autopsy material using ^{3}H-SCH23390 and ^{3}H-spiperone, respectively. Specific ^{3}H-spiperone (D_2) binding sites were selectively decreased in pallidum of HE patients (by 44%, $p<0.01$). Mn deposition in pallidum could explain the MRI signal hyperintensity in cirrhotic patients and Mn neurotoxicity with concomitant alterations of pallidal dopaminergic function could play an important role in the pathogenesis of extrapyramidal symptoms in human HE. (Funded by MRC Canada).

786.3

RELATIVE VULNERABILITY OF NIGRAL DOPAMINE AND GABA NEURONS *IN VIVO* TO INHIBITION OF SDH P.K. Sonsalla*, L.Manzino and G.D. Zeevalk. Neurology, UMDNJ-Robert Wood Johnson Med.Sch., Piscataway,N.J.08854

In an accompanying abstract, we demonstrate that mesencephalic dopamine (DA) neurons *in vitro* are relatively more vulnerable, as compared with GABA neurons, to a mild metabolic stress. Such findings may help explain the selective loss of DA neurons in Parkinson's Disease should a generalized mitochondrial defect be found to be an underlying cause of the disease. The following study was done to determine if the same heirarchy of sensitivity of nigral DA and GABA neurons to a mild metabolic stress was maintained *in vivo*. To test this, unilateral stereotaxic injections of varying amounts of malonate, (0.25-1μmol), a reversible inhibitor of succinate dehydrogenase (SDH), or vehicle were administered into the substantia nigra (SN) of the brains of 4 mo. old Sprague Dawley rats. One week following infusion, the animals were sacrificed and DA and GABA content in the left (injected) and right nigra and striata were measured by HPLC. Malonate caused a dose dependent loss of both nigral DA and GABA on the injected side. As expected, striatal DA (from nigral projections), but not GABA, was decreased in the ipsilateral striatum. Co-administration of succinate (1.5μmol) with malonate (0.5μmol) completely prevented loss of nigral DA and GABA and striatal DA, which indicates that the effects of malonate were mediated by inhibition of SDH. Nigral DA and GABA levels were equally vulnerable to malonate. The EC_{50} for loss of DA and GABA by malonate were 0.39 and 0.42 μmol/nigra, respectively. Tyrosine hydroxylase positive neurons were significantly decreased in the nigra on the injected side. The difference in *in vivo* versus *in vitro* findings may suggest that in nigra from normal animals, mild metabolic stress *per se* does not preferentially affect DA neurons. Other factors such as increased dopamine turnover or decreased glutathione levels have been suggested as contributing factors in Parkinson's Disease and may combine with a mild metabolic stress to enhance vulnerability of the DA population. These possibilities are currently being investigated in the laboratory. This work was supported by a grant from the National Parkinson Foundation.

786.4

RELATIVE VULNERABILITY OF DOPAMINE AND GABA NEURONS IN VITRO TO INHIBITION OF SDH AND EFFECTS OF NMDA RECEPTOR BLOCKADE G.D. Zeevalk*, E. Derr-Yellin and W.J. Nicklas. Neurology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, N.J. 08854

There is evidence that metabolic defects may be a causative factor in Parkinson's (PD) as well as other neurodegenerative diseases. It is unclear at present how a generalized mitochondrial defect might result in the selective loss of dopamine (DA) neurons, a major pathological feature in PD. Here we examine the relative vulnerability of DA and GABA neurons in mesencephalic culture to inhibition of succinate dehydrogenase (SDH) by 3-nitropropionic acid (3-NPA)or malonate, irreversible and reversible inhibitors, respectively. These inhibitors produce different severities of metabolic stress. Metabolic inhibition was induced in 12 day old cultures by a 24 hr treatment with 3-NPA (0.1-0.5mM) or malonate (10-50mM). Cell death was assessed after a 48 hr recovery period by simultaneous measurement of the uptake of ^{3}[H]-DA and ^{14}[C]-GABA. 3-NPA and malonate caused a dose-dependent loss of DA uptake (EC50 0.21 and 42 mM, respectively). Exposure to 3-NPA was equally detrimental to the GABA population, whereas malonate was without affect. To determine the severity of metabolic stress imposed by 3-NPA or malonate, lactate production was measured in 12 day cultures treated with 3-NPA or malonate for 3 hr. Lactate production was 78% higher in 0.5mM 3-NPA treated cultures and 18% higher in 50mM malonate treated cultures when compared with untreated controls. Additionally, a 3 hr treatment with 0.5mM 3-NPA was sufficient to reduce DA uptake by 84% when measured after 48 hr of recovery. A 3 hr exposure to 50mM malonate did not produce toxicity. These studies suggest that 3-NPA causes a greater inhibition of oxidative metabolism. The presence of the NMDA antagonist MK-801 (1μM) during 24 hr of 3-NPA or malonate treatment completely protected against DA and GABA loss with 50mM malonate or 0.25 mM 3-NPA and partially protected versus 0.5mM 3-NPA. These studies indicate that the DA neurons *in vitro* display a relative vulnerability to mild metabolic stress as compared with mesencephalic GABA neurons. Protection by MK-801 suggests that mild metabolic stress triggers a secondary excitotoxicity mediated by NMDA receptors. This work was supported by a grant from the National Parkinson Foundation.

786.5

TIME COURSE AND MORPHOLOGY OF MPTP-INDUCED NEURONAL DEATH. V. Jackson-Lewis*, M.W. Jakowec, R.E. Burke and S. Przedborski. Dept. of Neurol., Columbia University, New York, NY 10032.

Mechanisms responsible for MPTP-induced dopamine (DA) neuronal death remains unknown and in mice it is even unclear whether neuronal death does occur. *In vitro* studies suggest that MPTP kills neurons by apoptosis. Herein, we investigated whether MPTP induces DA neuronal death *in vivo* in mice and whether the mechanism is that of apoptosis. C57/bl Mice received different doses of MPTP and were sacrificed at different time points for analyses of tyrosine hydroxylase (TH) immunohistochemistry, silver staining, and Nissl staining within the mesencephalon. We found that MPTP induces neuronal destruction in the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA). The active phase of degeneration began at 12 hr postinjection and continued up to 4 days. During this period, there was a greater decrease in TH-defined neurons than in Nissl-stained neurons suggesting that MPTP can cause a loss in TH without necessarily destroying the neuron. Thereafter, neuronal counts by both techniques equalized and there was no further loss of DA neurons. Dying neurons showed shrunken eosinophilic cytoplasm and shrunken darkly stained nuclei. Double staining revealed degenerating neurons solely among TH positive neurons of SNpc and VTA. At no time point and at no dose of MPTP was apoptosis observed. In addition, *in situ* labeling revealed no evidence of DNA fragmentation. This study demonstrates that the MPTP mouse model replicates several key features of neurodegeneration of DA neurons in PD and provides no *in vivo* evidence that MPTP kills DA neurons by apoptosis.

786.6

INHIBITION OF BRAIN NITRIC OXIDE SYNTHASE PROTECTS AGAINST MPTP-INDUCED DOPAMINERGIC TOXICITY. S. Przedborski[1]*, V. Jackson-Lewis[1], R. Yokoyama[1] and T.M. Dawson[2]. [1]Dep. of Neurol. Columbia Univ., New York, NY 10032 and [2]Dep. of Neurosci. & Neurol. Johns Hopkins, Baltimore, MD 21287.

MPTP produces an experimental model of Parkinson's disease in a variety of animal species. The mechanism by which MPTP kills dopamine (DA) neurons is uncertain. Recent evidence has implicated nitric oxide (NO) in various neurotoxic paradigms. Thus, we have investigated whether NO is involved in MPTP-induced DA neurotoxicity *in vivo* in the mouse. C57/bl Mice received 50 mg/kg 7-nitroindazole (7-NI) to inhibit brain neuronal NO synthase (n-NOS) activity. Inhibition of n-NOS in the striatum peaked around 15 min and was still evident at 8 hr after 7-NI injection. To test the effect of n-NOS inhibition on MPTP-induced toxicity, C57/bl mice were injected with 7-NI 15 min prior to MPTP (20 mg/kg; i.p.) every 2 hr for 8 hr; mice received additional injections of 7-NI every 8 hr for 4 days following the last dose of MPTP. Mice were then sacrificed at different time points for analysis of striatal DA content by HPLC and for determination of degenerating substantia nigra pars compacta (SNpc) neuron number by silver staining. Consistent with our earlier report (Jackson-Lewis et al., 1995), this regimen of MPTP caused 94% reduction in striatal DA content and 65% destruction of SNpc neurons. In contrast, MPTP-injected mice pretreated with 7-NI showed only a 25% reduction in striatal DA content and no detectable degenerating SNpc neurons. Mice receiving 7-NI only did not show any changes in striatal DA levels. This study demonstrates that inhibition of n-NOS protects against MPTP-mediated DA damage in mice. This finding suggests that NO is involved in the MPTP neurotoxic mechanism.

786.7

THE OXIDATIVE NATURES OF DOPAMINE AND L-DOPA AND THE EFFECT OF O-METHYLATION. JW Miller*, J Selhub, JA Joseph. Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Dopamine (DA) and L-Dopa are readily oxidized to form potentially neurotoxic, pro-oxidant, reactive oxygen species which have been implicated in aging and age-related disease (e.g. Parkinson's disease). Recent evidence, however, indicates that under certain conditions, DA and L-Dopa can also serve as free radical scavenging antioxidants. In the present study, we used the Oxygen Radical Absorbance Capacity assay to demonstrate these apparent antithetical oxidative natures. In addition, we determined that O-methylation, the first step in catecholamine catabolism, produces compounds with oxidative properties different from DA and L-Dopa. The fluorescent protein porphyridium cruentum ß-phycoerythrin (ßPE) was exposed at 37 ^{0}C to either of two distinct oxidizing agents: 1. a peroxyl radical generator (2,2'-azobis[2-amidinopropane] dihydrochloride (AAPH)), or 2. $CuSO_4$. The ßPE loses its fluorescence as it is oxidized by these compounds with the rate of loss accelerated by pro-oxidants and slowed by antioxidants. Both DA and L-Dopa slowed the rate of ßPE fluorescence loss caused by AAPH in a dose-dependent manner, indicating that they acted as antioxidants. The O-methylated metabolites of DA and L-Dopa, 3-O-methylDA and 3-O-methyldopa, also slowed the rate of fluorescence loss, but to a lesser extent than DA and L-dopa, indicating that these compounds were weaker antioxidants under these conditions. Contrarily, DA and L-Dopa increased the rate of ß-PE fluorescence loss caused by $CuSO_4$, indicative of pro-oxidants. The O-methylated metabolites, however, did not increase the rate of $CuSO_4$-induced fluorescence loss, suggesting that O-methylation of DA and L-Dopa protects against their pro-oxidative effects under these conditions. These results demonstrate that DA and L-Dopa can act as either pro- or antioxidants depending on the type of oxidative stress, and suggest that O-methylation may protect against transition metal-induced catecholamine oxidation.

786.8

DOPAMINE NEUROTOXICITY: INHIBITION OF MITOCHONDRIAL RESPIRATION. D.Ben-Shachar*, R.Zuk & E.Klein, Rambam Medical Center, Fac. of Medicine, Technion, Haifa, Israel.

Abnormal dopamine metabolism is associated with several neuronal and psychiatric disorders. Oxidation of dopamine has been implicated as the main cause of its toxicity in brain. Indeed, icv injection of dopamine to rats caused a dose dependent mortality with LD_{50}=90uM. Norepinephrine was less toxic. Desferrioxamine prevented dopamine induced mortality. However we could not link dopamine toxicity to any of the conventional indicators of oxidative stress state, i.e. lipid and protein oxidation, glutathione peroxidase and reductase activities. Instead, dopamine inhibited the activity of mitochondrial NADH dehydrogenase activity with IC_{50}=8uM; that of Norepinephrine was twice as much. ATPase activity was not affected by both catecholamines. Dopamine induced inhibition of NADH dehydrogenase activity was only partially reversed by desferrioxamine while that induced by norepinephrine was not affected. The mechanism of dopamine interaction with mitochondrial NADH dehydrogenase will be further discussed. The results suggest that catecholamines can cause toxicity, not only by inducing a state of oxidative stress, but also via interaction with he electron transport system of the mitochondria. The latter was further supported by the ability of ADP+P_i to reverse dopamine induced inhibition of NADH dehydrogenase activity in a dose dependent manner.

786.9

EFFECT OF GABA, TAURINE AND FULLEROL-1 ON EXCITATORY AMINO ACID-INDUCED NEUROTOXICITY. [1]J.-Y. Wu, [1]X. W. Tang, [1]D. L. Deupree*, [1]J. Medina, [2]C.-Y. Yang and [3]L. Y. Chiang. [1]Dept. of Physiol. & Cell Biol., Univ. of Kansas, Lawrence, KS 66045, and [2]Dept. Med., Baylor College of Med., Houston, TX 77030; [3]Ctr. Condense Matter Sci., National Taiwan Univ., Taipei, Taiwan.

Primary cultures derived from 16-17 day fetal rat brain at 14 days in vitro (DIV) were used in L-glutamate-induced neurotoxicity (GNT) studies. Cultures were briefly exposed to glutamate or its agonists, e.g. kainic acid (KA) and N-methyl-D-aspartate (NMDA) for 5 min and GNT was determined 24 hrs later by measuring the release of lactate dehydrogenase (LDH) to the media. It was found that GNT was potentiated by GABA and taurine at low concentration (0.01 to 1 mM) to an extent of 50% and 100%, respectively. At high concentration, 25 mM taurine but not GABA, can effectively block GNT. The potentiation of GNT by GABA and taurine is believed to be due to their action on opening Cl^- channel since GNT as well as potentiation of GNT by GABA and taurine is greatly reduced by Cl^- channel blockers, e.g. picrotoxin, R(+)-IAA-94, niflumic acid. Fullerol-1, a C_{60} compound derivative, was found to be a potent blocker of GNT. A complete protection by Fullerol-1 against GNT induced by 0.5 mM L-glutamate was obtained at 50 μM. The mechanism of neuroprotective function of Fullerol-1 against GNT remains to be determined. (Supported by grants from Office of Naval Research; NIH, NS20978; and NSF, BNS-8820581)

786.10

TUBULIN IMMUNOSTAINING IN ORGANOPHOSPHORUS ESTER-INDUCED DELAYED NEUROTOXICITY (OPIDN). K.F. Jensen*[1], K.R. Wilmarth[2], J.K. Olin[1], M. Attia[2] & M.B. Abou-Donia[2]. [1]Neurotoxicology Div., HERL, US EPA, RTP, NC 27711 & [2]Dept. Pharmacology, Duke Univ., Durham, NC 27710

Cytoskeletal alterations occur in a variety of toxicant-induced axonopathies and may play a critical role in axonal degeneration. A single exposure to compounds such as diisopropyl phosphorofluoridate (DFP) can produce OPIDN, characterized by ataxia progressing to paralysis with a central-peripheral distal axonopathy. We previously demonstrated cytoskeletal aggregations, labeled by antibodies recognizing phosphorylated neurofilaments (PNF) and CaM kinase II (CaMKII), occur in axons early in OPIDN. The immunoreactivity for PNF and CaMKII is lost prior to the appearance of axonal varicosities characteristic of the late stages of OPIDN. To further characterize cytoskeletal alterations in this axonopathy, sections of spinal cord from control hens, and hens killed 3, 7, 10, 14 and 21 days after administration of 1.7 mg/kg DFP, were stained with a monoclonal antibody recognizing the alpha and beta subunits of tubulin. In control animals, the tubulin antibody labels the central core of axons, similar to PNF and CaMKII antibodies. The tubulin antibody also labels the cytoskeletal aggregations detectable in axons in early stages of OPIDN. But unlike PNF or CaMKII, tubulin immunostaining persists in many prominent axonal varicosities characteristic of late stages of OPIDN. These observations indicate that neurofilaments and tubulin are differentially altered in OPIDN.

786.11

EXCITOTOXIC-INDEPENDENT DNA FRAGMENTATION AND FREE RADICAL FORMATION PRODUCED BY 2-IMINOTHIAZOLIDINE-4-CARBOXYLIC ACID (2-ICA): A NOVEL HIPPOCAMPAL NEUROTOXIN. R. S. Bitner*, G. K. W. Yim and G. E. Isom. Dept. Pharmacology and Toxicology, Purdue University, W. Lafayette, IN 47097.

We previously demonstrated that 2-ICA, a minor metabolite of cyanide, produced seizures in mice that were qualitatively and quantitatively similar to seizures observed with glutamate and were blocked by glutamate antagonists. Unlike known glutamate neurotoxins, 2-ICA administered i.c.v. produced lesions primarily confined to the CA1 region of the hippocampus, similar to animal models of transient global ischemia (Bitner *et al.*, Neurotoxicol. 16:115, 1995). Failure of MK-801 or CNQX to protect against 2-ICA lesions suggested that a glutamate receptor-mediated mechanism was not involved in 2-ICA neurotoxicity. Because excitotoxicity is normally associated with delayed necrotic cell death, the goal of this study was to determine if 2-ICA produced apoptotic neuronal death, which recently has been implicated in CA1 lesions of transient global ischemia. Using a modified TdT dUTP nick end labeling (TUNEL) method, we showed that an i.c.v. injection of 2-ICA (3.2 μmol) in mice produced DNA fragmentation primarily in CA1 hippocampal neurons. We also found that 2-ICA (0.32-3.2 mM) increased the rate of formation of reactive oxygen species (ROS) in acutely dissociated neuronal culture using the fluorescent probe 2',7'-dichlorofluorescein. Consistent with an excitotoxic-independent action, the increased rate of formation of ROS by 2-ICA was not significantly reduced by MK-801 (1.0 μmol), but was blocked by the free radical scavengers, n-acetyl-L-cysteine (100 μmol) or catalase (100 U/ml). These results suggest that 2-ICA hippocampal CA1 damage may be due to DNA fragmentation caused by the formation of ROS and that 2-ICA may be useful in studying the role of apoptosis in hippocampal neurodegeneration. (Supported in part by NIEHS grant 04140).

786.12

ROLE OF A CALCIUM-ACTIVATED METALLOPROTEASE TRANSIN/STROMELYSIN IN PLASTICITY AND APOPTOSIS IN THE RAT BRAIN ?

M. Reeben, J. Arbatova, P. Riekkinen Sr and M. Saarma. Institute of Biotechnology, University of Helsinki, Helsinki, FIN-00014, Finland.

Transin/stromelysin is a neutral calcium-dependent metalloprotease (EC.3.4.24). It is known to be induced both on mRNA and protein level in rat pheochromocytoma PC12 cells during differentiation with nerve growth factor. As it degrades type IV collagen, laminin and fibronectin this suggests that transin may function to degrade the surrounding extracellular matrix during the invasive process of axonal elongation in neuronal development thereby allowing the movement of growth cones and axons toward specific targets. We were interested whether transin is expressed in adult brain and could it be regulated by neuronal activity ? As a model for neuronal activity we used seizures induced in rats by injection of glutamate analogue kainate or GABA receptor channel blocker metrazol (pentylenetetrazol). Kainic acid, but not metrazol-induced seizures lead to the induction of transin mRNA. Transin mRNA was induced in CA1 region of hippocampus, cortex, piriform cortex and amygdala region as revealed by *in situ* hybridization. The induction of transin mRNA by kainic acid was mediated by NMDA receptor and was dependent on protein synthesis. One of the possible plasticity related functions of this transin induction could be release of protease in the axon endings promoting sprouting induced by kainate. Another possibility is that transin induction by kainate may play role in neuronal damage. Differently from several other genes regulated by neuronal activity transin was not induced by metrazol and seizure activity induced by metrazol is known not to produce neuronal damage. Simultaneous DNA fragmentation in the brain regions of transin mRNA expression indicates the possible role of transin in apoptosis occuring during kainic acid-induced seizures. Transin mRNA is known to be regulated by *fos*-dependent and *fos*-independent pathways. Currently c-*fos* knockout mice are used to study the role of c-*fos* in kainic acid-induced transin mRNA induction.

ALZHEIMER'S DISEASE: MECHANISMS OF DEGENERATION II

787.1

REGIONAL SPECIFICITY OF INCREASED BASIC FIBROBLAST GROWTH FACTOR (FGF-2) IN ALZHEIMER'S DISEASE.E.G. Stopa*, V. Kuo-LeBlanc, M. Rodriguez-Wolf, M. Vitek, M. Kohls, A.M. Gonzalez and A. Baird. Brown University, Providence, RI, The Picower Institute,Manhasset,NY and The Scripps Research Institute, La Jolla, CA.

Basic fibroblast growth factor is increased in the prefrontal cortex and hippocampus of Alzheimer(AD) brains (Stopa et al.,BBRC 171:690-696,1990).Both of these regions are known to be severely affected in AD.It was therefore unclear whether bFGF would be increased in less affected areas such as the striatum.

In this study, we utilized immunodensitometry to quantitate bFGF in areas severly affected by AD: amygdala=a, A10,A20 and hippocampus=h,and areas which are less severely involved by the disease:A42 and striatum=s,in 14 control and 14AD patients.Immunodensitometry was also performed for synaptophysin and B amyloid.The immunocytochemical procedures utilized specific antibodies raised against bFGF(773), synaptophysin (Biogenex) and B amyloid (GE10).Densitometry measurements of reaction product were accomplished using NIH Image 1.51 and averaging data from 10 randomly selected 400X microscopic fields in each respective area. Immunodensitometry measurements for bFGF expressed as AD positive pixels/control positive pixels indicated a roughly 2-fold increase in affected areas A10=3.4,A20=2.8,a=2.1,h=2.2 vs less involved regions A42=1.24 and s=1.7. Immunodensitometry measurements for synaptophysin were consistent with synaptic loss in all brain regions: A10=.92andA20=.98 vs A42=.93and s=.97. Similar measurements of the amyloid burden indicated an increased deposition of Beta amyloid :A10=66.3,A20=21.0,a=33.0and h=41.7 vs A42=80.4and s=12.5.

These data indicate that bFGF is preferentially increased in brain areas severely affected by AD and suggest that this increase may be related to multiple factors including synaptic loss and B amyloid deposition.(AG10682)(DK18811)

787.2

DIFFERENTIAL BRADYKININ-INDUCED CA^{2+} ELEVATION IN FIBROBLASTS FROM ALZHEIMER'S VS. CONTROL DONORS. N. Hirashima†, R. Etcheberrigaray†, M. Racchi‡, S. Bergamaschi‡, F. Battaini‡, S. Govoni‡ and D.L. Alkon†‡* †*Laboratory of Adaptive Systems*-**NINDS**, National Institutes of Health, Bethesda MD 20892 and ‡Pharmacological Science Institute, University of Milan, Italy.

A number of studies have indicated that non-neural tissues of Alzheimer's Disease (AD) patients exhibit alterations at the cellular and molecular level. We have recently identified molecular alterations in fibroblasts from AD patients including: functional absence of a ≈ 113 pS TEA-sensitive K^+ channel, enhanced bombesin-induced Ca^{2+} release, and reduction of Cp20 (a memory-associated GTP binding protein). This study explores the bradykinin(BK)-IP_3 induced Ca^{2+} release in a new and extended population of AD and control donors. Fibroblasts were obtained from the Coriell Cell Repositories and from the Sacred Heart Hospital of Brescia, Italy. Cells were used three to four days after seeding, at comparable levels of confluence (≈ 60 to 80 cells per mm^2). Fluorescent images (fura 2-AM) at 340 and 380 nm were acquired at a rate of 2/sec. for 200 sec, with a Hamamatsu Argus 50 system. Stimulation with 100 pM BK elicited virtually no responses from control cells (N= 11 cell lines), with only one cell line responding (expressed as % of responding cells). In contrast, 12 out of 14 AD cell lines had clear responses, $p < 0.001$ (Mann Whitney). The difference between the two groups analyzed by a contingency table (response/no response) was also highly significant, $p < 0.0001$ (Fisher's exact test). Cell lines from "escapees" showed responses similar to those of control cell lines. BK concentrations of 1 and 10 nM, elicited similar responses in AD and controls. These observations confirm and extend our previous results indicating that IP_3-mediated Ca^{2+} release is enhanced in AD fibroblasts. In addition, these results provide another parameter whereby AD patients might be diagnostically differentiated from controls.

787.3

ALTERED PRESYNAPTIC PROTEIN NACP IS ASSOCIATED WITH PLAQUE FORMATION IN ALZHEIMER'S DISEASE. T. Saitoh*, A. Iwai, M. Mallory and E. Masliah Dept of Neurosciences, Sch. of Med., Univ. of California-San Diego, La Jolla, CA 92093

We have recently identified, in the brain tissue of patients afflicted with Alzheimer's disease (AD), the non-Aβ component of AD amyloid (NAC) as a new constituent of amyloid. NAC is derived from a larger precursor, NACP, a presynaptic protein. The semiquantitative immunoblotting demonstrated that the proportion of NACP/synaptophysin was more than double in the AD frontal cortex as compared to controls. NACP was localized to approximately 80% of the synaptophysin-immunoreactive boutons, presumably the presynaptic terminals, and to the dystrophic neuritic component of the plaques. Although the overall numbers of NACP-positive boutons were decreased, there was a significant increase in the intensity of NACP-immunoreactivity per bouton in AD. This increased intensity of immunoreactivity of boutons in AD was not observed with anti-synaptophysin, consistent with immunoblotting-based quantification. Antibodies against NAC immunoreacted with amyloid in 35% of the diffuse plaques and 55% of the mature plaques. Normal aged control brains containing small groups of diffuse plaques were negative with anti-NAC. Double-immunolabeling studies with Aβ antibodies showed that NAC immunoreactivity is more abundant in the center portion of amyloid than in the periphery. These studies suggest that there is a connection between metabolism of presynaptic proteins and amyloid formation, and that NAC might follow diffuse Aβ accumulation resulting in the formation of compact amyloid and mature plaques.

787.4

NACP, THE PRECURSOR PROTEIN OF NON-Aβ COMPONENT OF ALZHEIMER'S DISEASE AMYLOID, BINDS Aβ THROUGH THE HYDROPHOBIC REGION AND STIMULATES Aβ AGGREGATION.
M. Yoshimoto*, A. Iwai, D. Kang, D. A. C. Otero, Y. Xia, and T. Saitoh, Dept. of Neurosciences, University of California, San Diego, La Jolla, CA 92093-0624

NAC (Non-Aβ Component of Alzheimer's Disease Amyloid) is a peptide isolated from and immunologically localized to brain amyloid of patients afflicted with Alzheimer's Disease (AD). Molecular cloning of a cDNA encoding NAC has shown that NAC was derived from its precursor protein, NACP, of 140 amino acid residues. We found that NACP produced in *E. coli* bound with Aβ1-38 and Aβ25-35 immobilized on nitrocellulose, but did not bind with Aβ1-28 under the same conditions. The NACP binding to Aβ1-38 was abolished by the addition of Aβ25-35, but not by Aβ1-28, suggesting that the hydrophobic region of the Aβ peptide is critical to this binding. NACPδ, a deletion mutant of NACP lacking the NAC domain, did not bind with Aβ1-38. Furthermore, the binding between NACP and Aβ1-38 was decreased by the addition of peptide Y, a peptide which covers the last fifteen residues of NAC. These data indicate that NACP and Aβ peptides bind through the interaction between the C-terminal portion of NAC and the 25-35 region of Aβ peptides. In an aqueous solution, NACP formed an SDS insoluble complex with Aβ1-35 after incubation at 37°C for 72h, with a molar ratio of 1:1. Aβ1-38, on the other hand, formed an SDS insoluble aggregate when incubated with NACP at a ratio of 1:125 (NACP:Aβ), while Aβ1-38 alone or NACP alone did not aggregate under the same conditions. Thus NACP can bind Aβ peptides through the specific sequence and can promote Aβ aggregation. These data suggest that NAC or NACP may be involved in the process of amyloid formation from Aβ. This work has been funded by Alzheimer's Disease Research, a program of the American Health Assistance Foundation, and NIH grant AG05131.

787.5

INDUCTION OF HEME OXYGENASE-1 mRNA IN CEREBRAL VESSELS AND NEOCORTEX IN ALZHEIMER'S DISEASE.
D.R.D. Premkumar*, M.A. Smith, R.K. Kutty, B. Wiggert, G. Perry and R.N. Kalaria. Departments of Neurology and Pathology, Case Western Reserve University, Cleveland, Ohio and The National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA

Previous studies demonstrated the specific association of heme oxygenase-1 (HO-1) protein to the pathological lesions present in Alzheimer's disease (AD). In this study, we used reverse transcription -polymerase chain reaction methods to show the increased expression of HO-1 but not heme oxygenase-2 (HO-2) mRNA transcripts in cerebral cortex and cerebral vessels from subjects with AD compared to age-matched non-AD controls. Neither HO-1 nor HO-2 mRNA was altered in the cerebellar samples; a brain region usually spared from the pathological alterations of AD. There was no clear evidence that the expression of HO-1 in these tissues was related to postmortem interval, the age of subjects or any known premortem agonal factors. The specificity of our observations was further verified by the demonstration of unchanged ß actin mRNA transcripts but increased GFAP mRNA in tissues from AD subjects compared to controls. Our findings indicate the specific induction of HO-1 mRNA and increased expression of HO-1 protein in neocortex and cerebral vessels but not HO-2, and suggest that oxidative stress is important in the pathogenesis of AD. Supported by grants from NIA and ADRDA.

787.6

DECREASES IN CEREBRAL HEMOGLOBIN OXYGENATION DURING BRAIN ACTIVATION IN PATIENTS WITH ALZHEIMER'S DISEASE MONITORED BY MEANS OF NEAR INFRARED SPECTROSCOPY (NIRS) - CORRELATION WITH SIMULTANEOUS rCBF-PET MEASUREMENTS.
C. Hock*[1], F. Müller-Spahn[1], K. Villringer[3], H. Heekeren[2], A. Ghidau[2], R. Störmer[2], S. Schuh-Hofer[2], U. Dirnagl[5], A. Villringer[5], S. Minoshima[4], S. Ziegler[3], M. Schwaiger[3]. Depts. of Psychiatry, University of Basel[1], Switzerland, and Munich[2], Germany, Depts. of Nuclear Medicine, Technical University of Munich[3] and Ann Arbor[4], Michigan, Dept. of Neurology, Humboldt University of Berlin[5], Germany.

We applied a dual channel NIRS system using two pairs of optodes placed on the left parietal cortex and on the left frontal cortex to study non-invasively changes in cerebral hemoglobin oxygenation during activation of brain function in patients with Alzheimer's disease (AD) compared to controls. Healthy elderly subjects (n=8, age 60 ± 15) showed increases in oxygenated hemoglobin [HbO_2] as well as total hemoglobin [HbT] in both frontal (mean (arbitrary units) ± SEM, 2.00 ± 1.45 and 2.37 ± 1.99, respectively) and parietal cortex (1.26 ± 1.03 and 0.23 ± 0.88, respectively) during performance of a verbal fluency task. In contrast, AD patients (n=10, age 65 ± 13 years) showed decreases in [HbO_2] and [HbT] in the parietal cortex (-2.48 ± 0.91, $p<0.02$, and -3.37 ± 1.13, $p<0.05$, respectively) and, simultaneously, increases in [HbO_2] and [HbT] in the frontal cortex (1.40 ± 2.56 and 0.31 ± 3.30, respectively). Simultaneous NIRS and ($O15\text{-}H_2O$-) PET measurements during brain activation (stroop test, n=10) showed that PET rCBF changes were paralleled by changes in [HbT] as measured by NIRS. Modeling the spatial relationship of the NIRS-measurements to the PET-image revealed best correlation assuming a NIRS sample volume covering approximately the outer 1.6 cm of the brain cortex beneath the optodes (r=0.71, p=0.021). Regional decreases in cerebral [HbT] and rCBF during activation of brain function in patients with AD may contribute to the development and the time course of neurodegeneration.

787.7

^{31}P MRS *IN VITRO* STUDY OF GRADED HYPOXIA IN FISCHER 344 RATS. R.J. McClure*, K. Panchalingam, W.E. Klunk and J.W. Pettegrew. *Lababoratory of Neurophysics, University of Pittsburgh Medical Center, W.P.I.C., Pittsburgh, PA 15213.*

Episodes of mild to moderate energetic stress could, in some individuals, trigger molecular and metabolic events that result in the biochemical findings in the brains of Alzheimer's disease (AD) patients. In this study we determined the phospholipid and high-energy phosphate metabolite levels by ^{31}P MRS at 11.7 Tesla of perchloric acid brain extracts of Fischer 344 rats that had been exposed to graded hypoxia. New born, 5 and 10 day, 1, 2, 12, and 24 month old rats were studied after 0, 15, 30, 60, 120, 300, and 600 seconds of acute hypoxia. Levels of α-glycerol phosphate (α-GP) and inositol-1-phosphate were elevated after 30 seconds of hypoxia, the greatest effect was seen in young animals. α-GP is present in very low levels in new born to 10 day old animals. Phosphocreatine (PCr) levels were found to decrease after 30 seconds of hypoxia in all age groups. There is a link between phospholipid and high-energy metabolism which is seen in the linear correlation between PCr and α-GP levels. These correlations for the different age groups are: new born, 5 and 10 day ($p=0.001$, $r=-0.84$); 1 and 2 month ($p=0.0001$, $r=-0.92$); and over 12 month ($p=0.001$, $r=-0.75$). The slope of the linear regression line for these PCr and α-GP correlations increases with age which suggest that the older animals are more susceptible to energetic stress. This connection between energetic stress and alteration in membrane phospholipid metabolism may be relevant to the pathophysiology of AD.

787.8

POSTISCHEMIC DEPLETION OF GFAP REACTIVITY IN HIPPOCAMPAL ASTROCYTES IS PREVENTED BY HWA285
A.McRae[1]*, M. Knabel[2], P. Schubert[2], K. Rudolphi[3]
[1]University of Göteborg Sweden 41390, [2]Max Planck Institute of Psychiatry Martinsried Germany, [3]Hoechst AG Frankfurt a.M. Germany

Transient (5 min) forebrain ischemia causes a reactive hypertrophy of astrocytes in the gerbil hippocampal CA1 area, reflected by an increased immunoreactivity for glial fibrillary acidic proteins (GFAP) at the 2nd postischemic (p.i.) day. In animals developing complete nerve cell death, this is followed by a depletion of GFAP staining in the CA1 stratum radiatum which appears at the 7th p.i. day as a demarcated area with a complete loss of cellular and neuropil staining. We have previously observed that pre-treatment with the neuroprotective pharmacon propentofylline (HWA285) interferes with the initiation of the astrocyte reaction preventing the ischemia-induced increase of GFAP staining. However, daily post-treatment with HWA285, started at the 2nd p.i. day was found in the present study to prevent the consecutive fading of the GFAP reactivity completed at the 7th p.i. day in the CA1 area. Here, a maintained strong GFAP staining was observed in process-bearing astrocytes, whereas neuronal damage was not prevented (as verified by MAP2 staining). We have emerging evidence that chronic HWA treatment increases GFAP reactivity in cultured astrocytes and reinforces the development of potentially neuroprotective properties by strengthening the cAMP signalling, whereas microglial activation and APP immunoreactivity are suppressed. Such a pharmacological conditioning of glial cell may provide protection against progressive, but less dramatic neuronal death than induced by ischemia, e.g. in Alzheimer or Parkinson patients.

787.9

ALPHA-1 ANTICHYMOTRYPSIN IN HUMAN OLFACTORY BULB. RG. Struble* D. Bayley, M Ghobrial. Southern Illinois University School of Medicine. Springfield, IL. 62794

Alpha-1 antichymotrypsin (ACT) is associated with senile plaques and amyloid deposition, the classic markers for Alzheimer's disease (AD). One hypothesis in AD is that senile plaques are sites of abortive sprouting representing dysfunctional control of regeneration. We hypothesized that if ACT is involved in regeneration, we should find it in olfactory bulb of normal controls. The olfactory bulb is unique because the olfactory nerve regenerates continuously throughout the life of the organism. We therefore examined the immunohistochemical localization of ACT in olfactory bulbs in humans ranging in age from 1 year to 85 years. **We found that ACT-containing perikarya, probably glia, were consistently found in regions where axonal growth could be inferred.** ACT immunoreactive glia were distributed around the olfactory nerve as it entered to bulb and throughout the granule cell layer, both regions rich in GAP43. We conclude that ACT is present in sites of axonal growth. ACT's presence in senile plaques may represent localized, abortive sprouting. If this hypothesis is correct, then treatments to induce axonal growth may be counterproductive in arresting the progression of Alzheimer's disease. Supported by NIA grant #P30 AG08014.

787.10

CHRONIC EXPRESSION OF PLATELET ACTIVATING FACTOR (PAF) AND CLUSTERIN IS ASSOCIATED WITH NEURODEGENERATION IN RAT BRAIN FOLLOWING EPILEPTIFORM SEIZURES. Bennett, S.A.L.[1,2*], Tenniswood, M.P.R.[1], and Roberts, D.C.S.[2]. [1]Alton Jones Cell Science Center, Lake Placid, NY, USA, 12946; [2]Life Sciences, Carleton University, Ottawa, Ont., Canada, K1S 5B6.

Pro-inflammatory molecules are associated with the principal lesions of a wide variety of progressive neurodegenerative disorders. It is unclear whether induction of inflammatory response molecules in the central nervous system precedes lesion formation or is a consequence of chronic exposure to non-degradable plaque elements (i.e. Periodic acid Schiff (PAS) + material). In the present study, we have investigated the relationship between clusterin, PAF, and the development of PAS + lesions following limbic status epilepticus. Clusterin is a complement regulatory protein and a prominent marker of neurodegeneration and apoptosis. PAF is a potent pro-inflammatory autacoid implicated in mediating ischemic injury and convulsive activity. In the present experiments, epileptiform seizures were elicited in male Wistar rats by IP administration of 10 mg/kg kainic acid. Brain sections were processed at various time points after seizure induction for PAS histochemistry and clusterin, PAF, and PAF receptor (PAFR) expression by immunohistochemistry and *in situ* hybridization. Apoptosis was determined by *in situ* TUNEL labelling and by evaluating DNA fragmentation. RNA was extracted from unfixed tissue blocks and regional changes in PAFR and clusterin mRNA expression evaluated by Northern analysis and RT-PCR. Results demonstrate that epileptiform seizures induce both programmed cell death and PAS staining 24-168 hr after seizure onset. Of significant interest was the finding that changes in PAF and PAFR expression were correlated with subsequent alterations in clusterin expression, PAS staining, and apoptosis. Transient PAF and PAFR expression preceded transient clusterin expression in areas that demonstrated only limited cell loss. Chronic PAF and PAFR expression occurred in those areas expressing clusterin at later time points exhibiting substantial cell loss and PAS+ lesions. These data suggest that chronic exposure to pro-inflammatory agents precedes and may, in fact, contribute to the etiology of neurodegenerative lesions. (Supported by M.R.C.)

787.11

ANTI-MICROGLIA ANTIBODIES IN SERA OF ALZHEIMER´S PATIENTS. M.R. Lemke*, M. Glatzel, A. Henneberg. Dept. Psychiatry, Medical Center, D-85049 Ingolstadt, Germany (M.R.L.), Dept. Neurology, Ulm University, D-89070 Ulm, Germany (M.G.,A.H.)

Objective: Immune mechanisms seem to be involved in tissue destruction in Alzheimer´s disease (AD). There is evidence indicating the importance of microglia in AD. In brains of AD patients the presence of complement components has been documented, that are typically activated by immune complexes. Therefore, sera of AD patients were tested for antibodies against various brain structures.

Method: In patients (n=30) with AD (according to DSM-III-R and NINCDS-ADRDA criteria, MMSE<16) and matched controls (n=30, MMSE>24) serum antibodies were measured using an indirect immunofluorescence test as published previously.

Results: In nine patients perinuclear antibodies against microglia were found in amygdala and frontal cortex. Only one of the controls showed antibody binding to microglia.

Conclusions: The results support the hypothesis of involvement of immune mechanisms in AD. Perinuclear antibodies to microglia may play a role in tissue destruction of AD. Whether these findings are specific for AD or represent a subgroup of AD needs further evaluation.

787.12

EFFECT OF ATP DEPLETION ON THE ACTIVITY OF PK40^{erk2} IN DIFFERENTIATED PC12 CELLS: IMPLICATION FOR ALZHEIMER'S DISEASE. Y.Luo, R.Mehra, L.Butler, V.M.Ingram , Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Hyperphosphorylated TAU is a major constituent of tangles found in Alzheimer brains; ATP is depleted in such brains. We previously reported that the brain protein kinase PK40^{erk2} can hyperphosphorylate TAU to resemble Alzheimer TAU, as found in the tangles; it is strongly inhibited by free ATP in excess over MgATP. The mitochondrial uncoupler FCCP will deplete ATP levels in differentiated PC12 cells. (1) While in-gel kinase assays show that FCCP treatment abolishes the activities of 4 MBP protein kinases by an unknown mechanism, the same assay shows the activity of another kinase to be unaffected. This appears to be PK40^{erk2} by Western blot, and by in-gel assays with hTau40 or neurofilament proteins as substrates. The increased in-vivo activity of a neurofilament kinase reported by us [Bush et al.,PNAS 1995, 92:1861] is therefore likely to be due to up-regulation of the kinase in the cell by ATP depletion [release from partial inhibition]. The PK40^{erk2} assay of ERK2-specific immune-precipitates from extracts of treated cells also shows no in-vitro increase of kinase activity. FCCP does not increase synthesis or tyrosine phosphorylation of PK40^{erk2}. (2) FCCP treatment increases, mechanism unknown, the amount of protein phosphatase PP2A in extracts. This may account for the increase in the dephosphorylated TAU-1 epitope, reported by us earlier. The results explain the late onset of TAU hyperphosphorylation in Alzheimer disease and suggest a novel mechanism for the regulation of certain kinases.

LONG-TERM POTENTIATION: PHYSIOLOGY VII

788.1

ACTIVATION OF POSTSYNAPTICALLY SILENT SYNAPSES DURING PAIRING-INDUCED LTP. D. Liao* & R. Malinow, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY and *Dept. of Physiology & Biophysics, U. of Iowa, Iowa City, IA.

We have tested the hypothesis that in the CA1/subiculum region of hippocampus there exist synapses with only NMDA receptors (and thus effectively silent at resting potentials), and these synapses acquire AMPA responsivity after LTP. Consistent with this hypothesis, more synaptic failures were observed at hyperpolarizing (-65mV) than at depolarizing (+55mv) holding potentials (n=25, 52.7±4.6% Vs 20.0±2.7%). The difference in failure rate at -65mV and +55mV was blocked by 100 μM AP-5, (n=5). To stimulate just pure NMDA synapses, a (subminimal) stimulus can be set so that all trials produce failures at -65mV, but responses are seen at +55mV. We recorded 100 consecutive trials at -65mV with a subminimal stimulus. Pairing depolarization with the same subminimal stimulus produced subsequent synaptic responses at -65mV (12 of 22 experiments compared to 3 of 17 with no pairing; $p < 0.05$, Chi-test). In other experiments we could identify failures at hyperpolarizing and depolarizing potentials before and after LTP. After LTP there was a significant decrease in failure rate at hyperpolarized potentials, but no significant change at depolarized potentials (56±6% to 34±6% at -65mV vs 19±4% to 22±5% at +55mV; n=12).

788.2

LOW FREQUENCY STIMULATION INDUCES HOMOSYNAPTIC LTD IN THE CA1 REGION OF THE ADULT HIPPOCAMPUS IN VIVO. A.J. Heynen, M.F. Bear* and W.C. Abraham. Department of Psychology, University of Otago, Dunedin, New Zealand and Department of Neuroscience and HHMI, Brown University, Providence, RI 02912

Previous in vitro studies have demonstrated that low frequency stimulation (LFS; 1-5 Hz) of the Schaffer collaterals induces long term depression (LTD) of synaptic responses in CA1. This synaptic modification is input-specific, frequency-dependent, reversible and dependent upon N-methyl-D-aspartate (NMDA) receptor activation. Although homosynaptic LTD is now well characterized in hippocampal slices obtained from juvenile animals, it remains controversial whether LTD occurs in the CA1 region of adult hippocampus in vivo. The purpose of the present study was to examine LFS-induced LTD in the Schaffer collateral synapse in the intact preparation. Adult male Sprague Dawley rats (250-400 gm) under pentobarbital anesthesia (65 mg/kg) had recording and stimulating electrodes positioned in the stratum radiatum of one hemisphere. Field excitatory postsynaptic potentials (EPSP; 2-8 mV) were monitored for 30 minutes prior to application of LFS (900 pulses @ 1Hz). A lasting depression of synaptic responses was consistently observed following LFS (EPSP slope at 30 min post LFS = 84.1 ± 4 % of baseline; n = 9). LFS-induced LTD could be reversed by subsequent application of high frequency (100 Hz) stimulation. Repeated episodes of LFS saturated LTD (75.3 ± 3 % of baseline; n = 5). LTD was input-specific (homosynaptic), as responses to stimulation of afferents originating in the contralateral hemisphere (commissural input) remained unaffected by LFS of the Schaffer collateral input. Experiments in progress indicate that induction of LTD is frequency-dependent and requires NMDA receptor activation. Together, these results demonstrate that the properties of homosynaptic LTD observed in vitro also apply to the adult hippocampus in vivo. Supported in part by grants from the NZ Health Research Council.

788.3

HETEROSYNAPTIC LTP AND LTD OCCUR IN ASPINOUS CA1 INTERNEURONES. C. Stricker*, L. Reece, A.I. Cowan and S.J. Redman. Div. Neuroscience, John Curtin School of Med. Res., Australian National University, Canberra, Australia.

Synaptic specificity of LTP in CA1 pyramidal neurones has been attributed to compartmentalization of calcium in the spines associated with the activated synapses. This hypothesis was examined by testing for synaptic specificity at excitatory synapses on interneurones located in the CA1 pyramidal cell layer which are largely aspinous[1]. A standard hippocampal slice preparation from young rats (17-24 days) was used, maintained at 30°C, and bicuculline (10μM) was added to the ACSF. Intracellular recordings from interneurones were distinguished from recordings from pyramidal neurones by their short spike duration (< 1ms), pronounced spike afterhyperpolarization and lack of spike adaptation to a maintained current step. EPSPs were evoked from two stimulation sites in stratum radiatum: one near the cell body layer and the other adjacent to stratum moleculare. Stimulus strengths were adjusted to evoke EPSPs in the range 1-5 mV. EPSPs were evoked from each input at 0.5 Hz to obtain baseline responses. Independence of the two inputs was demonstrated by lack of occlusion when both inputs were stimulated simultaneously. One input was then tetanised at 100 Hz in five bursts of 20-40 stimuli while the stimulus was abolished from the other input. During conditioning the cell was depolarized until it discharged at a high frequency. Conditioned responses for both inputs were then recorded at 0.5 Hz for at least 30 mins. When one input was conditioned (n=21), the non-conditioned EPSP potentiated (10), depressed (7) or did not change (4). The conditioned EPSP potentiated (6), depressed (10) or did not change (5). The frequency of these various outcomes was independent of whether the conditioned input was proximal or distal from the soma. The heterosynaptic LTP observed in these interneurones supports the hypothesis that spines confer synaptic specificity to LTP in pyramidal cells.

[1]Schwartzkroin, P.A and Kunkel, D.D. (1985). *J. Comp. Neurol.* **232**, 205-218.

788.4

INDUCTION OF HEBBIAN AND NON-HEBBIAN LTP AT THE HIPPOCAMPAL MOSSY FIBER SYNAPSE BY DISTINCT PATTERNS OF HIGH FREQUENCY STIMULATION. N.N. Urban* and G. Barrionuevo *Dept. of Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.*

Long trains of high frequency tetanic stimulation (L-HFS, 100 pulses at 100 Hz) delivered to the hippocampal mossy fiber (MF) input to CA3 pyramidal cells induce a form of LTP not requiring post-synaptic activation, *i.e.* a non-Hebbian form of LTP. Specifically, MF-LTP induction by L-HFS is unaffected by blockade of ionotropic glutamatergic synaptic transmission during tetanus by 10 mM kynurenic acid (Kyn) (Castillo *et al. Neuron* **12**:261), postsynaptic membrane potential, calcium chelation and CA3 cell dialysis (Zalutsky & Nicoll, *Science*, **248**:1619, Katsuki *et al., Neurosci. Res.* **12**:393, Langdon *et al., J. Neurobiol.* **26**:370).

We reported (*Soc. for Neurosci. Abstracts* **20**:302.13) that brief-HFS (B-HFS, 8 pulses at 100 Hz, 10 times at 0.2 Hz) reliably induces an NMDA-independent form of MF-LTP that is inhibited by whole-cell dialysis. We hypothesized that MF-LTP induced by B-HFS represents a Hebbian form of LTP (Jaffe & Johnston, *J. Neurophysiol.* **64**:948). We now report that, in contrast to L-HFS, B-HFS fails to induce MF-LTP in the presence of Kyn. (Post B-HFS amplitude = 97% of control, N=15). MF-LTP was induced by B-HFS after wash out of Kyn (208% of control, N=13). This result suggests that B-HFS induced MF-LTP requires the activation of the postsynaptic CA3 cell. In 5 additional experiments, NMDA receptors were blocked by both 10 μM MK-801 and 25 μM D-APV, cells were whole-cell voltage clamped at -80 mV, and dialyzed for at least 25 min with BAPTA (1 mM) and CsF (120 mM). In these experiments, B-HFS induced LTP (161% of control) of the field EPSP but not of the simultaneously measured MF-EPSC (93% of control). Taken together these data demonstrate that both Hebbian and non-Hebbian forms of NMDA-independent LTP can occur at the MF-CA3 synapse; B-HFS induces a Hebbian form of LTP while L-HFS also can induce a non-Hebbian form of LTP. Supported by a Howard Hughes Medical Institute Predoctoral Fellowship and NS 24288

788.5

SELECTIVE RECRUITMENT OF AMPA RECEPTOR MEDIATED CURRENT DURING LTP IN MEDIAL PERFORANT PATHWAY OF RAT DENTATE GYRUS. **S. Wang* and J.M. Wojtowicz. Department of Physiology, University of Toronto, Toronto, Canada, M5S 1A8**

Whether long-term potentiation (LTP) is due to enhanced AMPA receptor mediated current alone or due to the enhancement of both AMPA and NMDA receptor mediated currents is controversial. In this study, we examined the medial perforant pathway synaptic transmission during LTP to address this issue. Whole-cell voltage clamp recordings were made in rat dentate granule neurons in a standard hippocampal slice preparation. Synaptic currents were evoked by stimulating the medial perforant pathway in the middle third of the dentate gyrus molecular layer. Membrane potential was held at -65 to -75 mV. Double exponential curves fitted to the decay phase of the responses revealed a large fast component and a small slow component. Using pharmacological agents we demonstrated that the small component, constituting 14 ± 7% (n = 8) of the total response, represents the NMDA current. LTP was induced by four brief trains of stimuli at 100 Hz, paired with postsynaptic depolarization to -20 mV. The success rate of inducing LTP in individual granule neurons was only 42% but the average magnitude was large. In a representative series of nine experiments the average potentiation was 339 ± 255%. During LTP expression, only the AMPA current increased while the NMDA current was unchanged. Furthermore, the variable magnitude of LTP was directly related to the relative size of the NMDA component prior to induction of LTP. (Supported by MRC of Canada)

788.6

THE EXTENT OF NMDA RECEPTOR ACTIVATION DURING A TETANUS DETERMINES WHETHER LTP IS EXPRESSED BY AMPA OR NMDA RECEPTORS.

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Analysis whether LTP is expressed by AMPA or by AMPA and NMDA receptors is a useful approach to determine if LTP is pre- or postsynaptically mediated, but has given contradictatory results. We have now tested the hypothesis that the threshold to induce LTP mediated by AMPARs differs from that required to trigger LTP expressed by NMDARs. Extracellular recordings were made in the CA1 hippocampal region in 0.3 mM Mg^{2+}.The NMDA component of the field EPSP ($fEPSP_N$) was isolated from the AMPA component ($fEPSP_A$) by adding CNQX before and 40 min after the tetanic procedure. Three tetanic stimulation were applied (after the washout of CNQX) at weak intensity, subthreshold for eliciting $fEPSP_N$) (T_W), or at strong intensity which elicited $fEPSP_N$ of 0.3 mV (T_S). T_W induced LTP of $fEPSP_A$ (59 ± 8%, n=9) but not of $fEPSP_N$ (11 ± 2%). In contrast,T_S failed to generate LTP of $fEPSP_A$ (9 ± 3%,n =9/13) but produced LTP of $fEPSP_N$ (89 ± 17 %). In the 4 remaining cases LTP of both components were observed. In presence of the redox reagent DTNB (200 μM) or 7-Cl^--Kyn (6 μM) which reduce $fEPSP_N$ by 50 %, T_S produced LTP of $fEPSP_A$ (63 ± 8 %, n=6) but not of $fEPSP_N$ (12 ± 4%). Under the same conditions, T_W generated LTP of $fEPSP_A$ of smaller amplitude than in control (30 ± 6 %, n= 8). We conclude that i) stronger activation of NMDA receptors during a tetanic train is required for inducing LTP of $fEPSP_N$ than for $fEPSP_A$; ii) there is a bell-shaped relationship between the extent of NMDA receptor activation during the train and the magnitude of LTP of $fEPSP_A$. Therefore, these findings reinforce the hypothesis that expression of LTP is a postsynaptic process.

788.7

NMDA-MEDIATED ACTIVITY-DEPENDENT SHORT-TERM PLASTICITY OF ELECTROTONIC COUPLING. Alberto Pereda* and Donald S. Faber *Dept. of Anatomy & Neurobiology, Medical College of Pennsylvania and Hahneman University, Philadelphia, PA.*

As reported previously discontinuous tetanic stimulation of the posterior eighth nerve produces a long-term homosynaptic enhancement of both the electrotonic and chemical components of the mixed excitatory postsynaptic potential (EPSP) recorded from the Mauthner(M-) cell lateral dendrite. We report here that the same tetanizing paradigm can evoke a shorter lasting potentiation (2-10 min.), which averaged 231.4 ± 24% (± SEM) for the electrotonic and 208.9 ± 20.4% for the chemical EPSPs (n=14) when measured right after tetanic stimulation. These modifications were not non-specific, since the M-cell antidromic spike, an indicator of the neuron's input resistance, did not change significantly (mean=100.5 ± 2.7% of control). The fact that short-term potentiation of electrotonic coupling occurs in parallel with that of the chemical EPSP suggested that these transient changes might have a presynaptic origin, for example, being due to an activity-dependent increase in the presynaptic calcium concentration. To test this possibility we compared the observed effects with those evoked by paired presynaptic stimuli. However, paired pulses failed to produce significant changes in electrotonic transmission (mean=102.2 ± 2.6% of control) although it strongly facilitated the second chemical response (mean=33.1 ± 7.8% of control, n=16). In contrast, the induction of this short term potentiation rather depended upon the discontinuous or burst-like property of the tetanizing paradigm, which optimizes NMDA receptor activation while continuous stimulation (50Hz) fatigues chemical transmission. Accordingly, the short-potentiation of both components required NMDA receptor activation, as the intermittent stimulus bursts failed to induce any potentiation in the presence of the NMDA receptor blockers APV and CPP (50-100μM, n=5). These potentiations were also blocked by intradendritic injections of the calcium chelator BAPTA (5mM, n=5). Thus, while paradigms known to raise the presynaptic calcium concentration were capable of only modifying chemical transmission, NMDA-mediated postsynaptic increases in calcium could evoke transient changes not only in the chemically mediated EPSP but also in electrotonic coupling.

788.8

PRESYNAPTIC LONG-TERM POTENTIATION (LTP) OF EXCITATORY SYNAPTIC TRANSMISSION IN SINGLE GRANULE CELLS (GCS).

G.Tong*, R.C.Malenka, C.E.Jahr and R.A.Nicoll. Dept. of Cellular and Molecular Pharmacology, Physiology and Psychiatry, University of California, San Francisco, CA 94143.

Previous studies in slices suggest that mossy fiber LTP in the hippocampal CA3 region is induced and expressed presynaptically by increased cAMP dependent protein kinase (PKA) activity in presynaptic terminals. We took advantage of microdot cultures to test whether single guinea pig GCs which form autapses show LTP. GCs were distinguished from other cells by their L-AP4 sensitivity. Consistent with the results from the slice preparation, tetanic stimulation (50 Hz, 1s, 4 times) in the presence of the NMDA receptor blockers D-AP5 (50 μM) and Mg^{++} (1 mM) induced a sustained potentiation of both NMDA and AMPA components of evoked excitatory postsynaptic currents (EPSCs) that lasted at least 30 min in L-AP4 sensitive cells. In contrast, in L-AP4 insensitive cells tetanic stimulation induced no LTP. Short applications (2 min) of forskolin (30 μM) also induced a long-lasting potentiation of evoked NMDA EPSCs in L-AP4 sensitive cells but a short-term potentiation (about 10 min) in L-AP4 insensitive cells. Furthermore, preincubating neurons with a PKA inhibitor, RP-8-CPT-cAMPS (100 μM) blocked tetanus induced LTP. Finally, the frequency of spontaneous miniature AMPA EPSCs was potentiated by tetanic stimulation but the amplitude of minis remained the same. These results suggest that in single GCs, tetanic stimulation causes a presynaptic increase in cAMP leading to a long-lasting enhancement of transmitter release. This enhancement may be due to an alteration of the internal machinery that controls transmitter release.

788.9

PAIRED-PULSE FACILITATION OF DUAL-COMPONENT EPSCS IN HIPPOCAMPAL PYRAMIDAL CELLS - IMPLICATIONS FOR LTP.
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The coefficient of variation of the AMPA receptor-mediated component of population EPSCs in CA1 cells is consistently larger than that of the NMDA receptor-mediated component (Kullmann, 1994; Neuron 12, 1111). I have proposed that this difference arises because AMPA receptors are absent or non-functional at a proportion of synapses, but can be activated with the induction of LTP. An alternative explanation, however, is that AMPA receptors are postsynaptic to release sites with a lower average release probability than NMDA receptors. If this were so, the paired-pulse facilitation ratio (PPFR) of AMPA receptor-mediated EPSCs should be higher than that of NMDA receptor-mediated EPSCs, since PPFR varies inversely with release probability. This has indeed been described under certain conditions (Clark et al, 1994; Exp. Brain Res. 101, 272). I have tested this hypothesis by measuring PPFR for the two components at the same synapses, by recording from CA1 cells with whole-cell electrodes in guinea pig hippocampal slices. EPSCs were elicited with paired stimuli in stratum radiatum (interval: 50 ms). The AMPA component was initially recorded at a holding potential of -80 mV, and the NMDA component was then recorded at +40 mV in 10 μM DNQX. No difference was seen between the PPFRs for the two components (t-test: $p>0.5$, $n = 18$). When two presynaptic stimulating electrodes were used, the PPFR often differed between the two pathways, but the same difference was seen whether the AMPA or NMDA component was studied. These findings imply that AMPA and NMDA receptors are exposed to glutamate released with the same release probability, and that the discrepancy between the coefficients of variation reflects a non-uniform distribution of clusters of functional AMPA receptors.

788.10

Tonic and phasic retrograde signals at CA3-CA1 hippocampal synapses
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Mechanism(s) of diffusive retrograde signalling at CA3-CA1 synapses and their importance for control and potentiated transmission are strongly debated. One of the main argument against this hypothesis is that LTP cannot be induced if the postsynaptic neuron is loaded with Ca^{2+} chelators, as nearby cells should still deliver diffusive signals. We investigated this problem in CA3-CA1 hippocampal cultures by monitoring miniature EPSCs (minis) (0.5 μM TTx present). Mini frequency LTP was elicited with 3 brief (200 ms) local applications of 90 mM KCl (388±37% potentiation at 50 min, n=9). LTP was prevented by loading postsynaptic neurons with either BAPTA (5-10 mM; 102±3% at 30 min, n=8) or NOS inhibitors (LNA 10 μM; 134±7% at 30 min, n=9) and by in phase application of hemoglobin (100 μM; 60 sec; 105±5% at 25 min, n=10). This strengths the idea that NO is a phasic retrograde signal required for LTP induction, but does not exclude possible additional BAPTA effects. We used a dual electrode recording approach to study the influence of postsynaptic BAPTA or NOS blockers on basal synaptic activity. Minis were monitored with a first patch pipette (WC perforated patch recording) before and after accessing the same cell with a second WC pipette to deliver compounds. Surprisingly, after the introduction of BAPTA, quantal frequency was drastically reduced (300±39% reduction, n=4) and this was not seen with NOS inhibitors (n=3). As in our culturing conditions autaptic connections are exceedingly low, we think that BAPTA also inhibits a tonic retrograde signal, which regulates quantal exocytosis and is presumably required for LTP. We conclude that presynaptic terminal functioning could be modulated by different tonic and phasic retrograde signalling mechanisms controlled by postsynaptic Ca^{2+}.

788.11

ACTION POTENTIALS PROPAGATING BACK INTO DENDRITES TRIGGERS CHANGES IN EFFICACY OF SINGLE-AXON SYNAPSES BETWEEN LAYER V PYRAMIDAL NEURONS
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Synaptic plasticity of single-axon (sa) synapses was examined using whole-cell recordings of synaptically coupled pairs of Layer V pyramidal neurons in the rat neocortex. Neurons were identified in slices with infrared differential interference contrasts microscopy. Simultaneous pre and postsynaptic depolarisation, that resulted in a burst of action potentials (APs), triggered a long-lasting (>40 min) increase in the amplitude of saEPSPs (n=9). APs triggered in synchrony in the pre and postsynaptic neurons by brief (5ms) current pulses and at rates from 10 to 40Hz also resulted in a long-lasting increase in synaptic efficacy (n=37) that was dependent upon the activation of NMDA receptors (n=8). Postsynaptic APs in the absence of synaptic input had no effect on saEPSP (n=6). Associative subthreshold depolarisation (n=8) or depolarisation to around -20mV in QX-314- loaded neurons (n=6) during synaptic input, also failed to induce an increase in synaptic efficacy. Indeed, strong associative depolarisation resulted in a reduction in the amplitude of most saEPSPs. The maximum increase in synaptic efficacy increased with frequency of synchronous pre-postsynaptic APs, while increasing the number of APs in a burst of 20Hz did not result in larger increases than that achieved with 2APs. Only saEPSPs that collided with the backpropagating AP increased in synaptic efficacy while saEPSPs that arrived 10ms after the backpropagating AP were either unchanged or weakened (n=6). These results show that the backpropagating AP is the trigger for changes in efficacy of this synapse. The constraints of AP frequency, number and timing suggest that connectivity only between pyramidal neurons that obtain common synaptic input will be enhanced which may enable the organisation of functional neuronal ensembles.

788.12

LTP INDUCED BY NITR-5 PHOTOLYSIS IN RAT CA1 HIPPOCAMPAL NEURONS WITHOUT PRESYNAPTIC ACTIVITY.
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A rise in postsynaptic $[Ca^{2+}]_i$ can trigger a long-lasting form of synaptic enhancement resembling LTP in rat hippocampal CA1 pyramidal cells (Malenka *et al.*, 1988). It is unclear whether this process is the same as LTP induced by afferent stimulation. Neither its interaction with tetanically induced LTP nor its requirement for presynaptic activity have been determined.

We used photo-activation of postsynaptically injected nitr-5 to elevate postsynaptic $[Ca^{2+}]_i$ to a level of about one-half to a few micromolar. This induced an increase in the rising slope of the EPSP obtained on stimulating Shaffer collateral afferents at 0.1 Hz. An increase of up to 140% was observed (average 66% ± 35, n = 14), and remained above baseline for the duration of long recordings (>40 min, n=7). Similar effects were obtained when afferent stimulation was suspended for 3 min before and after the photo-stimulus, indicating that presynaptic activity was not required for induction of this process.

When tetanically induced LTP (2-3 sets of 2 100-Hz tetani for 1-2 s separated by 20 s) of a test pathway preceded the photo-stimulus by 25-35 min, the photo-stimulus never increased the test EPSPs; control pathways were potentiated. Occlusion of photo-activated LTP by tetanic LTP suggests that they share common processes of induction and/or expression.

These results also suggest that at least a moderate level of LTP can be induced without involvement of presynaptic activity. This result has implications for models of LTP that require the concurrent action of a retrograde messenger with presynaptic activity. Supported by NIH Grant NS 15114.

PROCESS OUTGROWTH, GROWTH CONES, AND SPROUTING VII

789.1

DEVELOPMENT OF PRIMARY AFFERENT COLLATERAL BRANCHES IN THE EMBRYONIC MOUSE SPINAL CORD.
S. Ozaki* & W. D. Snider, Department of Neurology, CSNSI, Washington University School of Medicine, St. Louis, MO 63110.

The extension of collateral branches from growing axons to their target fields is a key event in the development of neural circuits. A favorable system for analysis is the collateral branching of primary afferents in the spinal cord which occurs over several segments from the point of entry of spinal axons. Here we characterize the development of primary afferent collateral branches in the mouse spinal cord as a framework for future studies in transgenic animals. Primary afferent projections were visualized using DiI crystals placed in the brachial plexus or thoracic nerves of mouse embryos aged between embryonic day (E) 10.5 and postnatal day (P) 0. The first primary afferent axons reach the spinal cord at E10.5 and grow rostrocaudally in the primordium of the dorsal funiculus. At this stage axons are relatively smooth and growth cones are prominent. There is a delay of at least 24 hours prior to elaboration of axon collateral branches into the spinal cord. Between E12.5 and E13.5 primary afferents develop nodes at frequent intervals, and collaterals enter the gray matter by branching from parent axons at the site of these nodes. There were many more nodes than collateral branches at all developmental stages. Nodes persisted at least until P0, the latest day studied. Analysis of semi-thin sections revealed only rare non-neuronal cells in the dorsal funiculus at the time collateral branching is initiated. The relationship between collaterals and radial glial cells is under study. We correlated the onset of collateral branching with expression of neurotrophin-3 (NT-3) mRNA in the embryonic spinal cord using *in situ* hybridization. NT-3 mRNA was expressed in the motor pools at E11.5 prior to the appearance of nodes or collateral branches, making it a candidate to regulate axon collateral branching in this system.

789.2

PRENATAL DEVELOPMENT OF SPINAL CORD CIRCUITRY INVOLVED IN URINARY BLADDER FUNCTION IN THE RAT.
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In an effort to understand how lumbar and sacral spinal cord circuitry controlling function of the urinary bladder develop during the prenatal period, a retrograde fluorescent labeling technique was used to identify and describe 1) autonomic and somatic motor neurons that provide innervation to bladder wall and sphincter musculature, and 2) the growth of vesicle (and related) primary afferents into the spinal gray matter. Crystals of the intensely fluorescent, lipid soluble tracer DiI were placed in the urinary bladders of aldehyde-fixed embryos that had been taken at different developmental stages (E13 through the day of birth) from timed-pregnant Sprague-Dawley rats. After an appropriate incubation period, the spinal cords were removed, sectioned, and examined by rhodamine epifluorescence microscopy.

By the day of birth, preganglionic parasympathetic neurons innervating the bladder have elaborated dendritic arborizations that extend medially to the central canal, into the contralateral spinal gray matter, and laterally into the lateral funiculus. Somatic motor neurons innervating sphincter musculature are located in the ventral-most regions of the spinal ventral horn and extend their dendrites in a medio-lateral plane and into the lateral and ventral funiculi. Primary afferents begin to enter the spinal gray matter on E15, and can be divided into two groups based on their morphology, 1) larger diameter fibers that enter the spinal cord more medially and descend to somatic motor neuron pools, and 2) smaller diameter fibers that enter the spinal cord more laterally and ramify densely within superficial (I and II) and deep (V and VII) laminae of the dorsal horn. The spatially- and temporally-patterned organization of the development of these neural elements suggests that, as in other systems, the development of spinal cord circuitry involved in bladder function may be regulated by the expression of centrally- or peripherally-derived trophic molecules.

789.3

TEMPORAL DIFFERENCES BUT SIMILAR DEVELOPMENTAL STRATEGIES HIGHLIGHT THE FORMATION OF AXONAL CONNECTIONS OF PERIRHINAL AND SOMATOSENSORY CORTICES. M.F. Barbe*, L. LaBarca-Meier, C. Leonard, K. Michihira, M. Salamon, P. Levitt. Dept. of Physical Therapy, Temple Univ., Phila., PA, 19140 and Dept. of Neurosci. and Cell Biol., UMDNJ, Robert Wood Johnson Medical School, Piscataway, NJ, 08854.

To investigate factors involved in the development of limbic and nonlimbic cerebral cortical circuitry, we examined the timing of thalamo-cortical and cortico-thalamic outgrowth in the somatosensory (Ss) and perirhinal (Prh) cortices. Lipophilic dye tracers, DiIC18-(3) or DiA were inserted into 4% paraformaldehyde fixed brains of rat fetuses aged embryonic day (E) 13-20. Seventy-eight rat fetuses were used. The lipophilic dyes were inserted into either presumptive Prh or Ss cortices, or the thalamus in single or double-labeling paradigms. The sections were examined for location of fibers extending from the insertion site and the presence and location of retrogradely labeled neurons. Results show that Prh cortico-thalamic axons meet the thalamo-cortical axons initially in the internal capsule on day E13.5 and probably represent the first axons from the cortex. Prh cortical axons arrive in the thalamus on E14-E14.5, a few days prior to axons from Ss cortex on E18. Axons from neurons located in lateral thalamus arrive in Prh cortex on E15 and in Ss cortex on E18, axons from neurons in midline thalamic region arrive in Prh on E18. Neurons labeled from either cortical site formed discrete clusters in thalamic areas. Axons segregated in the lateral portion of the internal capsule, with the Ss cortical-thalamic axons more dorsally situated than the Prh cortico-thalamic axons. These results show that while temporally distinct, limbic and nonlimbic cortical projections are initiated in similar, highly specific patterns. Supported by Temple University (M.B.) and NIMH grant 45507 to P.L.

789.4

EVIDENCE FOR MULTIPLE AXONS ON DEVELOPING DENTATE GRANULE CELLS. J.A. Schroer*, W. Yan, J.L. Young and J.H. Haring. Dept. of Anatomy and Neurobiology, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

We have collected a sample of nearly 200 neurobiotin-filled cells from rats aged PND14 to PND120. A small number of these neurons (<10%) exhibit two axons. The prevalence of multiple axons varies as a function of age being more frequent in younger rats, but is unrelated to experimental group. All neurons seem to have morphologies consistant with that of granule cells. One axon typically arises from the soma while additional axons take origin from either proximal or distal dendrites. Examples of both axons originating from the cell body exist. Both axons may be mossy fibers regardless of their site of origin or one axon may be a mossy fiber and the second assume a distribution similar to an interneuron. In older rats, mossy fibers are seen to originate from proximal dendrites in the absence of a second axon from the soma. Similarly, cells with an axon arising from a distal dendrite and projecting within the molecular layer have been seen. These data suggest that some granule cells may express a mixed axonal phenotype during maturation and that some of these cells will retain the atypical axon.

789.5

CLONING OF N16K, A NOVEL NEURAL DIFFERENTIATION-ASSOCIATED GENE AND DEVELOPMENTAL EXPRESSION. Y. Saito*, K. Maruyama#, T.C. Saido and S. Kawashima Dept. Molec. Biol., Tokyo Metropol. Inst. Med. Sci., Bunkyo-ku, Tokyo-113, #Lab.Neurochem., National Inst Physiological Sci, Okazaki, Aichi- 444

Mouse NS20Y cells can be reproducibly differentiated into neurons upon treatment with dibtyryl cyclic AMP (dbcAMP). In order to understand the molecular mechanism and cellular events which underlie neuronal differentiation, we constracted a subtractive cDNA library made from 48hr-treated NS20Y cells. We then screened randamly selected colonies by differential hybridization using labeled cDNA probes synthesized from the dbcAMP (−) and dbcAMP (+) mRNA pools. One clone, N16K, was markedly increased in NS20Y cells after dbcAMP treatment for 48hr. Expression of N16K mRNA in adult mouse was detected only brain and spinal cord, but not in other non-neuronal tissues. N16K encodes a protein sequence of 212 amino acids. Neither the nucleotide sequence of N16K nor the deduced amino acid sequence showed appreciable homologies to known sequences in the database. Futher characterization of N16K should provide additional information about its function during neuronal differentiation. (Suppoted by the Ministry of Education, Science and Culture of Japan and by Uehara Memorial Foundation)

789.6

EXPRESSION OF *WNT-7A* IN THE CEREBELLUM SUGGESTS ITS ROLE IN GRANULE CELL MATURATION. Patricia C. Salinas* and Fiona Lucas. Developmental Biology Research Centre King's College London WC2B 5RL U.K.

Wnt genes encode secreted proteins implicated in cell fate changes during development. To begin to define the specific cell populations in which *Wnt* genes act, we chose to examine *Wnt* expression in the cerebellum which has a relatively simple structure and contains well characterized cell populations. We found that *Wnt-7A* is expressed in the adult granular cell layer. *Wnt-7A* starts to be expressed from postnatal day 6 to 22 (P6 to P22) to then declines to low levels in adult cerebellum. From P22, *Wnt-7A* expression becomes restricted to the cerebellar lobules I to VIII, showing very low levels of expression in lobules IX and X. In this posterior region, called vestibularcerebellum, granule cells receive inputs from mossy fibers of vestibular origin. These results suggest that *Wnt-7A* expression may be down regulated by vestibular mossy fibers.

To determine the factors that control *Wnt-7A* expression, we analysed the agranular cerebellum of *weaver* mutant mice in which granule cells are present in the EGL at early stages but fail to migrate and then die in the EGL. *Wnt-7A* is undetectable in the EGL of *weaver* animals even though granule cells are still present at these stages as determined by the expression of the granule cell marker, *En-2*. Thus, granule cells from *weaver* fail to express *Wnt-7A* before they die. Moreover, *Wnt-7A* is ectopically expressed in the Purkinje cell layer of P6 and P15 animals and subsequently disappears. It is of interest that in *weaver* animals, mossy fibers, which normally will make synaptic contact with granule cells, form heterologous synapses with Purkinje cells. Granule cells exposed to *Wnt-7A*-expressing cells show an increased in the number and spreading of neurites. These results suggest a role of *Wnt* genes in the maturation of granule cell neurons.

789.7

DISTRIBUTION OF TWO PRIMARY PROTEIN KINASE C (PKC) SUBSTRATES, MYRISTOYLATED ALANINE-RICH C-KINASE SUBSTRATE (MARCKS) AND F1/GAP-43 mRNAs, IN THE ADULT RAT BRAIN. R. K. McNamara[1]*, D. G. Watson[1], & R. H. Lenox.[1,2,3] Departments of Psychiatry[1], Pharmacology[2], and Neuroscience[3], University of Florida College of Medicine, Gainesville, FL, 32610-0256

MARCKS (80-87kDa) and F1/GAP-43 (B-50/neuromodulin) are major substrates for PKC and important regulators of neuroplastic events during development as well as in the adult brain, where F1/GAP-43 mRNA levels remain elevated in regions associated with a high level of plasticity (Meberg & Routtenberg, 1991). The constitutive distribution of MARCKS mRNA has not yet been documented in brain. To determine the relative distribution of MARCKS and F1/GAP-43 mRNAs in the adult rat brain, male Sprague-Dawley rats (250-300 g) were sacrificed and perfused for *in situ* hybridization histochemistry. Serial sections were hybridized with radiolabeled antisense riboprobes corresponding to bases 354-1505 of the murine MARCKS cDNA (Seykora *et al.*, 1991) or 779-1295 of the rat F1/GAP-43 cDNA (Rosenthal *et al.*, 1987). Overall, MARCKS hybridization was expressed in the majority of regions but was most pronounced in pyriform ctx, medial habenular n., paraventricular hypothalamic n., and the cerebellar granule cell layer. Consistent with earlier reports, prominent F1/GAP-43 hybridization was observed in several regions including thalamic and hypothalamic n., lateral habenular n., entorhinal ctx, raphe n., and cerebellar granule cells. Within the hippocampal formation, MARCKS hybridization was highest in dentate gyrus granule cells, moderate in CA1 pyramidal cells, and low-absent in CA3 and hilar neurons, unlike F1/GAP-43 where hybridization was highest in CA3 and hilar neurons, moderate in CA1 pyramidal cells, and low-absent in dentate gyrus granule cells. While MARCKS and F1/GAP-43 mRNAs are co-expressed in certain regions (pyriform ctx/cerebellar granule cells), their differential expression in other regions (habenular complex/hippocampus) may reflect high levels of constitutive PKC activity, which has been shown to up-regulate F1/GAP-43 mRNA (Perrone-Bizzozero *et al.*, 1993) and down-regulate MARCKS mRNA (Brooks *et al.*, 1991). [Supported by NIMH grant MH50105]

789.8

COMPARATIVE EXPRESSION OF MYRISTOYLATED ALANINE-RICH C-KINASE SUBSTRATE (MARCKS) AND F1/GAP-43 mRNAs IN HIPPOCAMPAL GRANULE CELLS FOLLOWING KAINIC ACID. S. P. Baker[2]*, R. K. McNamara[1], D. G. Watson[1], & R. H. Lenox[1,2,3] Departments of Psychiatry[1], Pharmacology[2], and Neuroscience[3], University of Florida College of Medicine, Gainesville, FL, 32610-0256.

MARCKS (80-87kDa) and F1/GAP-43 (B-50/neuromodulin) are primary substrates of protein kinase C (PKC) and have been implicated in neuronal growth and differentiation by virtue of their localization in growth cone particles, association with the cytoskeleton, and changes in expression in cultured cells following exposure to differentiating agents like phorbol esters (e.g., Perrone-Bizzozero *et al.*, 1993). We have recently demonstrated that MARCKS mRNA is constitutively expressed at high levels in hippocampal granule cells (McNamara *et al.*, 1995), unlike F1/GAP-43 mRNA which is not constitutively expressed in granule cells (Meberg & Routtenberg, 1991). Kainic acid induces F1/GAP-43 mRNA in granule cells followed by the sprouting of their axons, the mossy fibers (McNamara & Routtenberg, *in press*). In the present study, we compared the expression of MARCKS and F1/GAP-43 mRNAs in granule cells at several points following kainic acid administration using *in situ* hybridization histochemistry. Male Sprague-Dawley rats (250-300 g) were administered either kainic acid (10 mg/kg, s.c.) or the drug vehicle (1 ml/kg) and sacrificed 6 hr, 24 hr or 5 d post-injection. Consistent with previous reports, F1/GAP-43 mRNA expression was induced in granule cells at 24 hr, but not 6 hr, and declined after 5 d when supragranular mossy fiber sprouting was observed in the ventral hippocampus. MARCKS mRNA expression remained at high levels in granule cells at each of the three time points. These data demonstrate that axonal outgrowth induced *in vivo* is preceded by an elevation of F1/GAP-43 mRNA in parent neurons whereas MARCKS mRNA expression appears to remain unchanged. These findings suggest that both F1/GAP-43 and MARCKS may be required for the initiation of axonal growth processes. The role of PKC-mediated posttranscriptional mechanisms in these events remains to be determined. [Supported by NIMH grant MH50105]

789.9

EXPRESSION OF B-50/GAP-43 IN ADULT OLFACTORY NEURONS IN TRANSGENC MICE RESULTS IN MORPHOLOGICAL CHANGES IN THEIR PROJECTIONS IN THE OLFACTORY BULB. A.J.G.D Holtmaat[1,2], P.A. Dijkhuizen[1], N.M.T. van der Lugt[3], A. Berns[3], H.J. Romijn[1], A.B. Oestreicher[2], F.L. Margolis[4], W.H. Gispen*[2], J. Verhaagen[1,2]. (SPON:Eur. Neurosci. Ass.) [1]Neth. Inst. Brain. Res., A'dam, NL; [2]Rudolf Magnus Inst., Utrecht, NL; [3]Neth. Cancer Inst., A'dam, NL; [4]Roche Inst. of Molec. Biology, Nutley, NJ, USA.

To study the function of B-50/GAP-43 *in-vivo*, we created transgenic mice, that express B-50/GAP-43 in the adult olfactory system. Using the olfactory marker protein (OMP) promoter, B-50/GAP-43 expression was directed to mature olfactory neurons, which do not normally express B-50/GAP-43. Mice bearing the OMP-B-50/GAP-43 transgene exhibited B-50/GAP-43 immunoreactivity in cell bodies, dendrites and axons of numerous mature neurons throughout the olfactory epithelium. This pattern of transgene expression is consistent with the action of the OMP promoter.We find that B-50/GAP-43 expression in mature olfactory neurons results in the formation of numerous hypertrophic OMP-positive primary olfactory axons with enlarged nerve endings. Confocal laser scanning microscopy on double-immunostained sections revealed that the mature olfactory axons often terminated in dilated grape-like structures which were preferentially located on the rim of the glomeruli. Double labelling with anti-tyrosine hydroxylase (TH)demonstrated that some OMP-positive olfactory neurons exhibit ectopic projections, between the TH-positive juxtaglomerular cells or associated with extra glomerular blood vessels. These phenomena were never observed in wild type litter mates and could be confirmed by Golgi staining of individual olfactory axon terminals. These data demonstrate that continued expression of B-50/GAP-43 in adult primary olfactory neurons *in-vivo* has a direct effect on the morphology and projection territory of their axon terminals and supports a role of this growth-associated protein in nerve fiber formation.

789.10

PROMOTER ELEMENTS CONTRIBUTING TO GAP-43 GENE REGULATION IN NEURONS. J.R. Weber, G.P. Hanes, and J.H.P. Skene*. Dept. of Neurobiology, Duke Univ., Durham, NC 27710.

Previous work in this and other labs has indicated that the 5' flanking region of the rat GAP-43 gene displays a significant degree of neural specific expression, and we now show in transgenic mice that 1 kb of this region can confer a significant degree of the temporal regulation of the GAP-43 gene. As one approach to elucidating the signaling pathways which control GAP-43 expression during axon outgrowth in developing or regenerating neurons, we are now identifying individual transcription factor binding sites. We have focused on a 386 bp subfragment of the 1 kb region that drives strong expression of a reporter gene in transiently transfected neurons from developing rat cerebral cortex, but has little or no activity in non-neuronal cell lines. Deletions within this 386 bp promoter indicate that a fairly small region located downstream of the TATA box consensus sequence is required for expression in neurons. Gel shift assays have identified three sequences which bind nuclear proteins from developing rat cerebral cortex: an AP1 consensus sequence and two apparently novel sites, Cx1 and Cx2. Mutation of all three of these binding sites eliminates the majority of activity from the 386 bp promoter in cortical cultures. In contrast, gel shifts with liver nuclear extracts have shown a strong binding activity (Liv1) which overlaps with, and would likely preclude binding to, Cx1 and Cx2. These studies have identified a small cluster of transcription factor binding sites, the AP1/Cx region, which is likely to play an important role in the regulation of the GAP-43 gene in neurons. Supported by NIH grant EY07397.

789.11

ROLE OF HIGHLY CONSERVED 3' UTR SEQUENCES IN THE GAP-43 mRNA IN RNA STABILITY. N. I. Perrone-Bizzozero*, R. Thompson, K-C. Tsai, V.V. Cansino, D. T. Kohn, T. Roham, and R. L. Neve. Dept. Biochemistry, Univ. New Mexico Sch. of Med., Albuquerque NM, 87131 and McLean Hospital, Belmont, MA 02178.

We have previously shown that the mRNA for the neural-specific, growth-associated protein GAP-43 is selectively stabilized during neuronal differentiation (J. Cell Biol. 120, 1263-1270, 1993). Given the absence of known instability-conferring elements in this mRNA, we explored the role of highly conserved sequences in its 3' untranslated region (3' UTR) in mRNA stability. Between the rat and chicken GAP-43 mRNAs, the 3' UTRs show 78% of sequence identity, a level that is similar to the conservation of their coding regions. In mRNA stability assays in transfected rat PC12-N36 cells, both the rat and chicken mRNAs decayed with half-lives of about 3.5 h. The GAP-43 3' UTR was also found to destabilize the otherwise stable globin mRNA, with the half-life of the chimeric transcript comparable to that of the wild type GAP-43 mRNA. Thus, highly conserved sequences within the 3' UTR are the major determinants of GAP-43 mRNA stability. The rat and chicken GAP-43 mRNAs were also tested in RNA-binding assays with brain cytosolic proteins. Both RNAs bound equally well to the three GAP-43 mRNA binding proteins from rat brain. Also, S100 proteins from chicken brain were able to bind rat GAP-43 mRNA sequences. Based upon these results, we propose that highly conserved sequences in the 3' UTR of this mRNA contribute to the control of GAP-43 gene expression via specific RNA-protein interactions. *Supported by NIH (NS-30255, GM-08139) TSGH-NDMC, Taipei, and RAC-1090, UNM Sch. Med.*

NEUROTROPHIC FACTORS: EXPRESSION AND REGULATION VII

790.1

IMMUNOHISTOCHEMICAL LOCALIZATION OF CILIARY NEUROTROPHIC FACTOR RECEPTOR α EXPRESSION IN THE RAT. A.J. MacLennan*, E.N. Vinson, L. Marks, M. Pfeiffer and N. Lee. Department of Neuroscience, University of Florida, Gainesville, FL, 32610-0244.

Ciliary neurotrophic factor (CNTF) decreases the death of neurons induced by natural causes, axotomy or genetic mutation. Molecular cloning and heterologous expression studies indicate that CNTF produces most, and possibly all, of these effects by binding to a protein referred to as CNTF receptor α (CNTFRα). Genetic "knockout" of this protein leads to significant developmental abnormalities. We used synthetic peptides corresponding to regions of the CNTFRα to raise and affinity-purify anti-CNTFRα polyclonal antibodies. The antibodies identify CNTFRα throughout the adult and developing central and peripheral nervous systems. Particularly high levels of expression were found in the olfactory bulb and the cell bodies and dendrites of cranial and spinal motor neurons, cortical pyramidal neurons, cerebellar purkinje cells and hippocampal neurons. Elevated levels were also detected in the axons of adult and developing peripheral nerves.

790.2

ONTOGENESIS OF RAT FETAL PREPROINSULIN I AND II mRNA AND INSULIN RECEPTOR mRNA WITHIN THE NERVOUS SYSTEM. R. Schechter*, D. Beju and T. Gaffney. William K. Warren Med. Res. Inst., Univ. of Okla. Health Sci. Ctr., and Dept. of Neonatology, Saint Francis Hospital, Tulsa, OK 74136.

We investigated the presence of preproinsulin I and II (PPI, PPII) mRNA within the fetal rat brain, spinal cord and dorsal root ganglia (DRG). We also studied the presence of the alpha chain of the insulin receptor (IR) mRNA in the same tissues. Total RNA was obtained from 15, 17 and 19 days gestational age rat fetal brain, spinal cord and DRG. Pancreas served as positive control for PPI and PPII mRNA, and liver for IR mRNA. Total RNA was subjected to reverse transcriptase template-specific PCR (RS-PCR) and RNAse protection assay (RPA) for PPI and PPII. IR was studied using RS-PCR. RS-PCR demonstrated a product of 381 base pair (bp) in brain, spinal cord and DRG in the 15, 17 and 19 days gestation that comigrated with the pancreatic product showing the presence of mRNA for PPI and PPII within these tissues. Semi-nested PCR products of 330 bp were obtained and further characterized with restriction enzyme digestion by HinfI and RsaI. The predicted fragments of 140 and 190 bp were obtained with HinfI for PPII, and fragments of 118 and 212 bp with RsaI for PPI. RsaI cuts only PPI, and HinfI cuts only PPII. In solution hybridization using a 32P-cRNA for PPI or PPII, followed by RPA showed bands from the 15, 17 and 19 days gestational age brain, spinal cord and DRG that comigrated with hybridized RNA from pancreas and with probe alone. RS-PCR also showed a fragment of 550 bp for IR as predicted within the brain, spinal cord and DRG for all ages studied. Fetal brain tissue was also prepared to localize insulin by electron microscopy (EM). EM showed insulin immunoreaction within the endoplasmic reticulum(ER), Golgi, cytoplasm, and cells processes. This study showed that preproinsulin I , preproinsulin II, and insulin's receptor are present within the fetal central and peripheral nervous system during nervous system development. Thus, the presence of the PPI and PPII mRNA and the immunoreaction within the ER and Golgi strongly indicate the de novo synthesis of insulin within the central and peripheral nervous system.

790.3

INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS (IGFBPs) IN MOUSE SPINAL CORD DURING DEVELOPMENT. **J.Y. Ma*, I.V. Smirnova, M.N. Zoubine, M.E. Avery, S. Huibao, P.M. Arnold, B.A. Citron and B.W. Festoff. Neurobiology Research Lab., VA Med. Ctr., Kansas City, MO 64128; Dept. of Neurology, Univ. of Kansas Med. Ctr., Kansas City, KS 66160.**

IGFs are paracrine and autocrine neurotrophic factors, and both IGFs and their receptors are expressed in the mammalian spinal cord. IGFBPs are soluble polypeptides which bind to IGF to form complexes and modulate bioactivity of both IGF-I and IGF-II. Our previous studies suggested that IGF signaling system is involved in motoneuron axonal regeneration and sprouting after sciatic nerve injury. IGFBPs have been well studied in the brain during development, but the expression of IGFBPs in the spinal cord during development has not been reported. With SDS-PAGE, followed by transfer to nitrocellulose membranes and Western ligand blot with ^{125}I-IGF-II, we analyzed IGFBPs in mouse spinal cord samples during embryonic (E14, E16) and postnatal (P3, P5, P10, P15 and P30) development. An approximate 30 kDa band was found. The highest level of this IGFBP is in E14, then gradually reduces, and is barely detected after P15. Our results demonstrate a major form of IGFBP with approximate molecular weight of 30 kDa in the spinal cord is developmentally regulated. The high level of this IGFBP in an early embryonic stage suggests a potential role of the IGFBP in the spinal cord development. (Supported by Cephalon, Inc., the ALS/Spinal Cord Research Fund and the Medical Research Service of the Department of Veterans Affairs)

790.4

GDNF mRNA IS FOCALLY EXPRESSED IN DEVELOPING MOUSE LIMB BUD IN REGIONS WHERE AXONS FORM THE PLEXUS D.E. Wright*, R.W. Gerfen, M.T. Saxena, J.C. Harding, L. Zhou and W.D. Snider. Dept. of Neurology, CSNSI, Washington University., St. Louis, MO, 63110.

The neurotrophic factor GDNF is a potent survival factor for motoneurons and is synthesized by Schwann cells and muscle. To further explore whether GDNF influences motoneurons during the early stages of peripheral innervation, we performed *in situ* hybridization on mouse embryos as motor axons enter the periphery. A mouse GDNF cDNA was PCR-amplified to synthesize 33P-labeled riboprobes. Similar to rat and human, two splice variants were amplified; the longer fragment was used to generate riboprobes. At later embryonic stages (E14-E16), GDNF mRNA was detected in non-neuronal cells along peripheral nerve and developing muscle. However at earlier stages (E10-E13), GDNF mRNA was expressed at high levels in a restricted region in the proximal limb bud. This focal expression was not observed in thoracic regions. Comparison with embryos labeled with DiI to reveal peripheral projections indicated the GDNF expression corresponds to areas where axon fascicles converge to form the plexus. The origin and identification of the cells expressing GDNF is currently being investigated. Other areas of intense GDNF expression at E11-E13 included mesodermal tissue surrounding the developing GI system and urogenital ridges. This expression may be relevant to the survival of peripheral neurons in these areas. Because GDNF belongs to the transforming growth factor (TGF)-ß superfamily, we compared GDNF expression to the patterns of related family members, including TGF-ß1, TGF-ß2 TGF-ß3, BMP-4, and BMP-6. Neither BMP-4 or BMP-6 mRNAs were detected in regions relevant to peripheral axon growth. TGF-ß1, TGF-ß2, and TGF-ß3 mRNAs were expressed in mesodermal regions adjacent to motor and sensory axons by E11. However, the expression patterns of the TGF-ßs were diffuse as compared to the focal expression of GDNF mRNA. Our results reveal that members of the TGF-ß superfamily are developmentally expressed in patterns consistent with an ability to influence axons in the periphery. The focal expression of GDNF mRNA in regions where axons converge to create the brachial and lumbar plexus suggest that GDNF may sustain motoneurons as their axons rearrange within the plexuses.

790.5

CHROMOSOMAL LOCATION AND ALTERNATIVELY SPLICED FORMS OF HUMAN GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR (GDNF). D.L. Choi-Lundberg[1,*], D.A. Figlewicz[1,2] and M.C. Bohn[1]. Depts. [1]Neurobiol. & Anat. and [2]Neurology, Univ. of Rochester Med. Ctr., Rochester, NY 14642.

GDNF is a potent neurotrophic factor for lower motor neurons and midbrain dopaminergic neurons and has neurotrophic activity on several populations of peripheral neurons. It is widely expressed in rat nervous system and peripheral organs throughout development and in the adult[1], and has been detected by reverse transcription-polymerase chain reaction (RT-PCR) in the adult human nervous system[2].

To identify the human chromosome on which the GDNF gene resides, DNA from a panel of 24 somatic cell hybrids containing one human chromosome (1 to 22, X or Y) in a hamster or mouse background was amplified by PCR with primers specific for exon 2 of human GDNF. A product of the predicted size was obtained only from the somatic cell hybrid containing human chromosome 5.

Two alternative forms of GDNF mRNA were identified in human fetal astrocytes by RT-PCR with primers that span the entire cDNA sequence. These were cloned into the SrfI site of the pCR-Script SK(+) vector and sequenced. One form contains a 78 base pair (bp) deletion corresponding to 26 amino acids of the pro region of the preproprotein. The second form has a novel 79 bp sequence which replaces 78 bp of the full length GDNF cDNA deleted in the first form, resulting in a frame shift and predicted early termination of the protein product. Initiation at another AUG codon could result in a protein product lacking a signal sequence for secretion but still containing full-length mature GDNF protein. Supported by NIH grant ES01247 and MDA. [1]Choi-Lundberg, D.L., and Bohn, M.C., *Dev. Brain Res.*, 85 (1995) 80-8. [2]Springer, J.E., Mu, X., Bergmann, L.W., Trojanowski, J.Q., *Exp. Neurol.*, 127 (1994) 167-70.

790.6

ALTERNATIVE GDNF TRANSCRIPTS IN RAT BRAIN: WIDESPREAD DEVELOPMENTAL AND REGIONAL EXPRESSION. D.O. Dean*, J. Alberch†, C.F. Dreyfus & I.B. Black. Dept. of Neuroscience and Cell Biology, RWJ Medical School, UMDNJ, Piscataway, NJ 08854 and †Dept. Biologia Cel.lular i Anatomia Patologica, University of Barcelona, Spain.

Parkinson's Disease is characterized by selective degeneration of midbrain dopamine neurons. Glial cell line-derived neurotrophic factor (GDNF) has been shown to enhance the survival and differentiation of these neurons. Previously, our laboratory has found multiple GDNF-related transcripts in rat and human. We now report the developmental and regional expression of GDNF and its alternative forms in rat brain.

Using RT-PCR, we examined GDNF-related transcripts in striatum and nigra dissected on embryonic day 17 (E17), postnatal day 1 (P1) and postnatal day 32 (P32). We identified three forms; GDNF, ATF-1 and ATF-3. In striatum, GDNF and ATF-1 message levels remained constant from E17 to P32. ATF-3 peaked at P1. In nigra, GDNF and ATF-1 message levels declined from E17 to P32, whereas ATF-3 was slightly elevated at P1. The relative persistence of GDNF and ATF-1 mRNA in striatum and reduction in GDNF and ATF-1 message in nigra suggest that these forms may code for target-derived trophic factors into adulthood.

To further explore region-specific expression, we examined cerebellum, cortex, hippocampus and septum. mRNA levels of all three transcripts peaked at P1 in cortex, hippocampus and septum. In cerebellum, GDNF, ATF-1 and ATF-3 message levels were very high at E17 and thereafter slowly declined.

In sum, we have observed differential developmental and regional expression of GDNF-related transcripts. The patterns of expression of GDNF and ATF-1 in nigra and striatum are consistent with the model of a target-derived trophic factor. ATF-3 may be important perinatally, as its expression at P1 is elevated in all regions, including striatum. The high expression of GDNF-related transcripts in cerebellum suggests a potential role in the development of this structure (see Mount *et al.*, this meeting). Supported by NICHD 23315 and Ministerio de Educacion y Ciencia.

790.7

EXPRESSION OF TRKB BUT NOT BDNFmRNA IN PARVALBUMIN CONTAINING NEURONS OF THE ADULT RAT VISUAL CORTEX. A. Cellerino, A. Burkhalter°*, L. Maffei and L. Domenici. Istituto di Neurofisiologia C.N.R. and Scuola Normale Superiore, 56127 PISA (Italy), °Dept Ana Neurobiol, Washington University Medical School, St Louis, MO 63110.

In the present study we analysed the distribution of trkB and BDNF in the adult rat visual cortex, focusing on parvalbumin (PV) positive cells, the major contingent of GABAergic neurons. Using antibodies to trkB (Santa Cruz Biotech, CA) we have found staining in both pyramidal and non pyramidal neurons. Double labelling experiments showed that most PV stained non pyramidal neurons co-localized trkB. Pyramidal neurons positive for trkB were surrounded by PV labeled boutons. Using an mRNA riboprobe for BDNF (nucleotides 147-657) and trkB (extracellular domain, nucleotides 19-412) we performed a double in situ-immuno histochemistry. We found that most PV stained cells contained trkB mRNA. Interestingly, co-localization of BDNF mRNA in PV stained non pyramidal cells was extremely rare. The present data suggest that BDNF, synthesized by pyramidal cortical neurons (Wetmore et al. Exp. Neurol. 109: 141-152, 1990), may act as postsynaptically derived neurotrophic factor or neuromodulator on PV neurons of the adult rat visual cortex.

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790.8

EXPRESSION PATTERN OF FIBROBLAST GROWTH FACTOR-9 IN DIFFERENT REGIONS OF THE RAT BRAIN DURING DEVELOPMENT. Alex T. Ho*, Candace Andersson, Karl A. Kuzis, Felix P. Eckenstein. Department of Cell Biology and Anatomy, Oregon Health Sciences University, 3181 SW Sam Jackson Park Rd., Portland, OR 97201.

The members of the fibroblast growth factor (FGF) family may regulate various aspects of CNS development. Analysis of FGF receptors expression during embryonic brain development and *in vitro* experiments with cultured neuroepithelial cells, have suggested that FGFs may function as regulators of neuronal and astrocytic differentiation during the patterning of the CNS. Recent isolation and cloning of a heparin-binding growth factor from a human glioma cell line have introduced a new member of the FGF family, FGF-9. We are testing whether FGF-9 may play a role during the development and the patterning of the rat brain. We have constructed sets of PCR primers based on the published FGF-9 sequence and performed a qualitative measurement of FGF-9 expression in the developing rat brain. Furthermore, we have cloned the PCR products in order to create probes for a quantitative measurement of FGF-9 using northern blot analysis and RNase protection assays. Our PCR and northern blot data suggest that FGF-9 is expressed in the rat CNS as early as embryonic day 12. Brain distribution analysis of adult and postnatal day 1 rat brain have shown that FGF-9 expression is concentrated in the cerebellar and the spinal cord regions of the rat CNS. Currently we are culturing specific cell populations from different parts of the brain to determine which cell types in the brain express FGF-9. Our data support the hypothesis that FGF-9 may play an important role in the differentiation of these regions during the development of the rat brain.

790.9

PRENATAL ONTOGENY OF EGF RECEPTOR AND TGF α mRNAs IN RAT BRAIN. H.I. Komblum[1],*K.Tatsukawa[1], R.J. Hussain[1], C.M. Gall[2], D. Lee[3], and K.B. Seroogy[4], [1]Depts. Pharm. and Peds. UCLA, LA, CA 90095 , [2]Anat. & Neurobiol. UC Irvine, [3]Lineberger Cancer Ctr, UNC, Chapel Hill, and [4]Anat. & Neurobiol. U. Kentucky.

Transforming growth factor alpha (TGFα) is related to epidermal growth factor (EGF) and acts at the EGF receptor (EGF-R). EGF and TGFα have been demonstrated to induce neuronal differentiation and neural stem cell proliferation *in vitro*. In the present study, we examined the ontogeny of EGF-R and TGFα mRNAs during fetal development of the rat brain by *in situ* hybridization, using ^{35}S-labeled cRNAs. EGF-R mRNA was present in the ventral midbrain by E13 and was detectable within the neuroepithelia of the midbrain, anterior pons and the posterior hypothalamus by E15. The cerebellar external granule layer was also densely labeled at this age. By E17, labeling was detectable in germinal zones of the telencephalon, including those of the septum, basal ganglia and amygdala. In the brainstem and diencephalon, labeled cells were also observed at a distance from the neuroepithelial surface. By E19, hybridizing cells were present throughout most germinal zones and multiple brain areas, in a pattern similar to that found on the day of birth. TGFα mRNA was expressed in the ventral midbrain and brainstem on E13. By E15, hybridization was apparent in the ventral telencephalon, thalamus, and caudal hypothalamus. By E17, hybridization densities were very high throughout many structures of basal telencephalon and within multiple thalamic and hypothalamic nuclei. Moderate hybridization densities were apparent throughout much of the brainstem, a pattern present on the day of birth. The present study demonstrates that the distributions of EGF-R and TGFα mRNAs are overlapping during fetal development, and that the TGFα/EGF-R system may be important in the normal genesis and/or differentiation of neurons and glia.

790.10

POSTNATAL EXPRESSION OF EGF RECEPTOR mRNA IN PROLIFERATIVE ZONES OF RODENT BRAIN. K.B. Seroogy[1]*, S. Numan[1], L.A. Opanashuk[1], C.M. Gall[2], D.C. Lee[3] and H.I. Komblum[4]. [1]Dept. of Anat. & Neurobiol., Univ. of Kentucky, Lexington, KY 40536, [2]Dept. of Anat. & Neurobiol., Univ. of California, Irvine, CA 92717, [3]Lineberger Comp. Cancer Ctr., Univ. of N. Carolina, Chapel Hill, NC 27599 and [4]Depts. of Pharmacol. and Pediatrics, UCLA, Los Angeles, CA 90024.

We have recently demonstrated that epidermal growth factor receptor (EGF-R) mRNA is prominently expressed in two principal germinal zones of the postnatal rat brain, the forebrain subventricular zone and the cerebellar external granule layer (EGL) (Seroogy et al., *Brain Res.* 670: 157-164). These results, together with *in vitro* data of others, suggest a role for an EGF-R ligand in regulating the proliferation and/or differentiation of neural progenitors in these regions. To determine if expression of EGF-R is a characteristic feature of additional brain proliferative zones, the *in situ* hybridization localization of EGF-R mRNA was examined in developing postnatal rat and mouse brain using an ^{35}S-labeled cRNA probe. Expression of EGF-R mRNA was also examined in proliferative regions of transforming growth factor-alpha (TGFα)-deficient mice. In addition to prominent labeling of the subventricular zone and EGL, in neonatal brain hybridization of the EGF-R cRNA was localized mainly to dorsal aspects of the neuroepithelia of the third ventricle, cerebral aqueduct and medullary central canal. Dense labeling was also present throughout the neocortical neuroepithelium. Hybridization densities declined substantially during the early postnatal period such that, by adulthood, reduced levels of EGF-R cRNA labeling were detected only in the forebrain subventricular region. In TGFα knockout mice, EGF-R mRNA expression was altered in the forebrain subventricular zone. These results indicate that most germinal zones of the developing brain express EGF-R mRNA and that expression of the receptor may be regulated by its endogenous ligand TGFα. Supported by the American Parkinson Disease Association (K.B.S.), MH10806 (S.N.), AG00538 (C.M.G.), CA43793 (D.C.L.) and the Dana Foundation (H.I.K.).

790.11

EXPRESSION OF HEPARIN-BINDING EPIDERMAL GROWTH FACTOR (HB-EGF) mRNA IN RAT BRAIN. L.A. Opanashuk*, S. Numan, and K.B. Seroogy. Department of Anatomy & Neurobiology, University of Kentucky, Lexington, KY 40536.

Two epidermal growth factor receptor (EGF-R) ligands, transforming growth factor-alpha (TGFα) and EGF, have recently been shown to promote survival and biochemical differentiation of neural populations from several brain regions. Although the distribution of TGFα and EGF-R mRNAs in brain has been described, the presence of EGF-R ligands other than TGFα is currently unresolved. In the present study, *in situ* hybridization was used to determine whether HB-EGF, a recently discovered member of the EGF family of mitogenic polypeptides, is expressed in the developing and mature central nervous system (CNS). Rat brain coronal sections were processed for the *in situ* hybridization localization of HB-EGF mRNA at neonatal and adult ages using an ^{35}S-labeled cRNA probe. The antisense HB-EGF cRNA was transcribed from a pBluescript II KS vector containing a 258 base-pair fragment derived from the 5' end of the cloned rat HB-EGF cDNA (cDNA kindly provided by Dr. Judith Abraham, Scios Nova). Initial results have demonstrated HB-EGF cRNA hybridization within several cortical regions including the piriform cortex, hippocampus, and throughout middle layers of the neocortex. In hippocampus, labeling was most prominent in the dentate gyrus. Several thalamic nuclei, including the ventrolateral geniculate nucleus and the ventrobasal complex, exhibited HB-EGF cRNA labeling. In brainstem, expression was detected in the dorsal raphe nucleus, area postrema, and caudal ventrolateral medulla. Dense hybridization was also present in the Purkinje cell layer of the cerebellum. HB-EGF mRNA levels were substantially higher neonatally than in adulthood suggesting that expression is developmentally regulated. These results raise the possibility that the novel EGF-R ligand HB-EGF may serve functional roles in select regions of the developing and adult brain. S.N. was supported by NIMH fellowship MH10806.

790.12

COEXPRESSION OF HEPATOCYTE GROWTH FACTOR, ITS RECEPTOR (*c-met*), AND TISSUE-TYPE PLASMINOGEN ACTIVATOR IN MURINE BRAIN. D.P. Thewke, W. Sather* and N.W. Seeds. Neuroscience Prog. & Dept. of Biochem/Biophy/Genetics, Univ. of Colorado HSC, Denver, CO 80262.

Hepatocyte growth factor/scatter factor (HGF) has pleiotropic effects on cells expressing its receptor, the *c-met* proto-oncogene product. HGF is synthesized as an inactive precursor which must undergo proteolytic cleavage to become biologically active. Both tPA and uPA are capable of activating HGF. As part of a study on the roles of tPA in the CNS, the expression of HGF, *c-met* and tPA in the murine brain was investigated. In situ hybridization reveals several regions of the developing and adult brain where the expression of HGF and/or *c-met* is coincident with tPA expression. Most notably in the cerebral cortex, the hippocampus, and the olfactory bulb. During development of the cerebral cortex, HGF mRNA is observed in the ventricular and subventricular zones, while *c-met* and tPA expression are observed in the cortical subplate and marginal zone. In the postnatal brain, *c-met* mRNA is detected in layers II-III and V of the cerebral cortex and the CA1 region of the hippocampus, while HGF mRNA is most prominently observed in the ependymal linings of the ventricles and the CA2 region of the hippocampus. Low levels of tPA mRNA are observed throughout the cortex, and high levels in all regions of the hippocampus. In the adult olfactory bulb HGF is expressed in the glomerular layer (GL), *c-met* in the olfactory nerve (ONL) and mitral cell layers (MCL), and tPA in the ONL, GL, MCL and the granule cell layer. These results suggests that tPA may have a role in the development and maintenance of these structures via activation of HGF in the immediate vicinity of its receptor. (Supported in part by NIH grant NS-09818)

790.13

GDNF: A MEMBER OF A GENE FAMILY EXPRESSED IN MANY SUBPOPULATIONS OF ADULT BRAIN NEURONS. N. A-M. Pochon[1], A.D. Zurn[1], A. Menoud[1], C. Bader*[2] and P. Aebischer[1]. [1]Gene Therapy Center and Surgical Research Division, Lausanne Univiversity Medical School, [2]Division for Clinical Neuromuscular Research, Geneva University Hospital, Switzerland.

Glial cell line-derived neurotrophic factor (GDNF) was first described as a potent trophic factor expressed ubiquitously in embryonic, but not in adult rat brain. We report here that GDNF belongs to a gene family whose members are widely expressed in adult rat brain. To identify the members of the GDNF family, total RNA from adult brain has been extracted and analyzed by Northern blot and RT-PCR. In each case, several messengers larger than the GDNF mRNA have been identified with a GDNF riboprobe. With an RNAse protection assay on total RNA of the adult cerebellum, two bands are clearly protected with a Sp6 GDNF riboprobe. Furthermore, several homologous genes have been amplified by PCR from genomic DNA, using different primers and temperature conditions.

In adult rat brain, the expression of the GDNF family is neuron- specific, as shown by in situ hybridization with a DIG riboprobe. The signal intensity observed in the different brain regions suggests that there are subpopulations of neurons expressing homologous members of the GDNF family. The hybridization signal is high in the hippocampus and a neuronal subset of the striatum. Using in situ hybridization and immunocytochemistry, dopaminergic neurons of the substantia nigra synthetizing mRNAs homologous to GDNF have also been identified. In conclusion, the members of the GDNF family have a tissue specific expression and seem to have a high sequence homology.

790.14

Expression of CNTF: new perspectives revealed by a lacZ reporter gene introduced into the CNTF gene locus.

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Although exogenously-applied ciliary neurotrophic factor (CNTF) exerts survival and differentiation effects on a variety of neuronal and glial cells in vitro and in vivo, the role played by endogenous CNTF is still incompletely understood. The high post-natal expression of CNTF in the PNS and its up-regulation after injury in the CNS has led to the proposal that CNTF may function as part of the response to damage in the nervous system. The levels and the sites of expression of CNTF during development and in adulthood are partially contraversial. To resolve these questions we have introduced the lacZ reporter gene into the CNTF coding region by homologous recombination in ES cells. Mice generated from such cells showed transient expression of the reporter gene in the developing embryonic nervous system. In the adult CNS reporter gene expression was detected in sub-populations of cells in most brain regions. In attempting to correlate the CNTF-promoter driven lacZ reporter gene expression with CNTF mRNA and protein expression patterns, we observed major differences in the expression levels of CNTF mRNA between rat and mouse in certain brain regions. Experiments are underway to identify the cells expressing CNTF.

790.15

IMMUNOLOCALIZATION OF NEUROTROPHIN NT4/5 AND ITS HIGH AFFINITY RECEPTOR TRK B IN THE RAT OLFACTORY BULB. J. C. Paz[1], J. N. Kott*[1], D. R. Kaplan[4], L.E. Westrum.[1-3] Depts. of Neurological Surgery[1], Biological Structure[2], and Psychology[3], Univ. of Washington, Seattle, WA 98195; ABL-Basic Research Program, NCI-FCRDC, Frederick MD.[4]

TrkB is the preferred high affinity receptor for the neurotrophin NT4/5 and exists in a truncated noncatalytic and full-length catalytic isoforms. Using immunohistochemistry, we are examining the developmental localization of NT4/5 and trkB in the rat olfactory bulbs (OB) of postnatal day 1, 5 (PN-1, PN-5) and adult animals. The OB is an ideal model in which to study the innervation/reinnervation process due to the unique regenerative capacity of the olfactory nerves (ON) throughout adult life. Immunoreactivity for truncated trkB remained moderate to strong throughout development in the ON and glomeruli (GL), while both full-length catalytic trkB and NT4/5 immunoreactivity were non-existent at PN-1, and strong at PN-5 and adult ON and GL. As labeling for both growth factor and receptor is found in the same fiber population, these findings may contrast with the classical theory that target-derived neurotrophins elicit trophic actions after retrograde transport back to the cell soma. The increased levels of NT4/5 and full-length catalytic trkB during the later stages of development may play a role in an autocrine and/or paracrine regulated neuronal maintenance rather than a guidance mechanism. The observation that catalytic trkB is expressed less strongly than truncated trkB suggests that truncated trkB may sequester excess NT4/5, thus limiting the diffusion of these growth factors. Supported by NIH grant NS09678 and a grant from NCI, DHHS, under contract N01-C0-4600. LEW is an affiliate of CDMRC.

790.16

DEVELOPMENTALLY REGULATED EXPRESSION OF *TRKA* AND *CHAT* IN THE RAT CAUDATE-PUTAMEN. Y. Li, D. O. Clary, L. F. Reichardt and W. C. Mobley*, Department of Neurology and Howard Hughes Medical Institute, UCSF, San Francisco, CA 94143.

TrkA, the receptor tyrosine kinase for NGF, is expressed in basal forebrain cholinergic neurons (BFCNs); its expression in development is regulated by endogenous NGF (Li et al., 1995, J Neurosci 15: 2888-2905). In the present work, we examined *trkA* expression in the developing caudate-putamen. Using double immunostaining, trkA was localized exclusively to caudate-putamen cholinergic neurons (CPCNs). TrkA mRNA was first detected at PD4 by in situ hybridization histochemistry. Northern analysis showed that trkA mRNA increased steadily from a low level at PD4 to the maximal adult level. The temporal pattern for the increase was similar to that for ChAT mRNA. By immunostaining, trkA protein was detected at PD0. Western blot showed that trkA protein increased during development and followed the pattern for the mRNA. Examining tyrosine phosphorylation of trkA in caudate-putamen triturates, we found that NGF activated these receptors at PD0 and that receptor activation was robust in older subjects. Infusion of NGF increased trkA and ChAT mRNA, as well as the size of trkA-positive neuron cell bodies. Our results indicate that trkA expression is highly regulated in CPCNs; they point to a role for NGF in CPCN morphological and biochemical differentiation.

Supported by NIH grants NS24054 and AG08938.

790.17

ACIDIC FIBROBLAST GROWTH FACTOR IS EXPRESSED BY BASAL FOREBRAIN AND STRIATAL CHOLINERGIC NEURONS. J.L. Bizon[1], J.C. Lauterborn[2], R.C. Elliott[2]*, and C.M. Gall[1,2]. [1]Dept. of Psychobiology, and [2]Dept. of Anatomy and Neurobiology, Univ. California, Irvine, CA 92717.

The basal forebrain cholinergic system is thought to be critical for the maintainance of cognitive abilities in both humans and rodents, and is susceptible to age- and injury-related degeneration. Recently, immunoreactivity for acidic fibroblast growth factor (aFGF), a substance which reportedly supports cholinergic neurons, has been localized in basal forebrain. In the present study *in situ* hybridization was used to evaluate the distribution and degree of colocalization of mRNAs for aFGF and the cholinergic marker choline acetyltransferase (ChAT) in rat forebrain. Neurons expressing aFGF mRNA were codistributed with ChAT-positive neurons throughout all fields of basal forebrain including the medial septum/diagonal band region and the striatum. Cells labeled by the aFGF cRNA also were distributed in regions not containing cholinergic neurons including lateral septum and several thalamic nuclei. Using double-labeling *in situ* hybridization, high levels of colocalization were observed in the medial septum, diagonal bands of Broca, magnocellular preoptic area and nucleus basalis of Meynert. In these fields over 85% of the cholinergic cells expressed aFGF mRNA. In striatum fewer cholinergic neurons contained aFGF mRNA and regional differences were observed. Specifically, among the ChAT-mRNA positive cells, 64% in caudate, 32% in ventral striatum (including nucleus accumbens) and 25% in olfactory tubercle expressed aFGF mRNA. These data demonstrate substantial, regionally specific, patterns of aFGF/ChAT mRNA colocalization and support the hypothesis that aFGF is an autocrine neurotrophic factor for cholinergic neurons in basal forebrain and striatum. Supported by AG00538 to C.M.G.

790.18

Expression and Regulation of Neurotrophic Factors in Basal Forebrain Oligodendrocytes *in vitro* and *in vivo* P. Qu*, X. Dai, H.Wu, W. J. Friedman, I. B. Black and C. F. Dreyfus. Dept. Neuroscience & Cell Biol., UMDNJ / Robert Wood Johnson Medical School, Piscataway, NJ 08854

Previous work in our lab indicated that: (1) neurotrophic factors are expressed in cultured basal forebrain oligodendrocytes: NGF and BDNF mRNAs were detected by solution hybridization, and BDNF, NT3 and NT4/5 proteins by immunocytochemistry; and (2) the expression of BDNF mRNA can be regulated by KCl as a depolarizing signal. In order to further investigate the characteristics of the expression and regulation of neurotrophic factors under physiological conditions, and the effect of different neurotransmitters, enriched basal forebrain oligodendrocyte cultures were treated with 10 μM glutamate for 48 hours. In contrast to the effect of KCl, which increases BDNF mRNA expression, glutamate treatment caused a decrease of BDNF mRNA to nearly undetectable levels. These preliminary data suggest that different types of depolarizing signals regulate neurotrophin expression in basal forebrain oligodendrocytes via different mechanisms. To evaluate the expression of neurotrophin proteins in basal forebrain *in vivo*, immunocytochemistry has been performed to co-localize neurotrophins with the oligodendrocyte markers A2B5, MAG and MBP. Preliminary results revealed overlap of the anti-NT3 positive cells with anti-A2B5, suggesting the presence of NT3 protein in the basal forebrain oligodendrocyte precursors. Ongoing studies will determine whether NT3 is also co-localized with MAG and MBP *in vivo*. In sum, our results suggest possible mechanisms for regulating neurotrophin expression by depolarizing signals *in vitro* and the existence of neurotrophin proteins in basal forebrain oligodendrocytes *in vivo*.

790.19

SECRETONEURIN IS EXPRESSED IN DEVELOPING RAT VENTRAL PALLIDUM, ACCUMBENS, AMYGDALA AND OTHER FOREBRAIN STRUCTURES.

H. Traurig*[1], W. Kaufmann[2], J. Marksteiner[2], S.K. Mahata[3], H. Mahata[3], R. Fischer-Colbrie[3] and A. Saria[2]. [1]Anatomy and Neurobiology, Univ. of Kentucky, Lexington, KY 40536. [2]Neurochemistry Unit, Psychiatry Institute & [3]Pharmacology, Univ. of Innsbruck, A6020, Austria.

Secretoneurin(Sn), an endoproteolytic peptide derivative of secretogranin II(Sg)(aa154-186), located in large, dense core vesicles, is released from neural tissue following capsaicin or potassium stimulation. Previous data revealed intense Sn immunoreactivities(I) in adult rat forebrain, hypothalamus, amygdala, hippocampus and brain stem.

The present study examined expressions of Sn-I and SgmRNA in rat brain on fetal(F) or postnatal(P) days F14,16,18,20, P1,5 and 10. In situ hybridization probe was complementary to rat Sg mRNA (nucleotides 193-240). Sn-I were compared to substance-P- and calbindin-I.

Sn and SgmRNA signals were first detected in basal forebrain and diencephalon on F16. By F20 components of the extended amygdala (bed nuc. of stria terminalis, nuc. accumbens and centromedial amygdala) and hypothalamus showed prominent Sn-I and SgmRNA signal. Sn and SgmRNA expressions were most evident in the postnatal hypothalamus. Sn-I and SgmRNA were also expressed in basolateral amygdala and dentate gyrus interneurons.

In conclusion, components of the extended amygdala and other brain structures express Sn-I and SgmRNA at specific stages of fetal and neonatal brain maturation. These expressions probably reflect development of circuits that link limbic and striatal functions.

(Supported by:Austrian Science Foundation grant #F00206)

791.1

FUNCTIONAL HETEROGENEITY OF $GABA_A$ RECEPTOR-MEDIATED RESPONSES IN THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS IN MALE AND FEMALE NEONATAL RATS. S.T. Smith,*[1] A.S. Clark[2], J. A. Lally, and L. P. Henderson[1]. [1]Depts. of Physiol. and Biochem., Dartmouth Medical School, [2]Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Gonadal steroids act during a critical period extending from late fetal stages to approximately postnatal (PN) day 10 to induce permanent sexual dimorphisms in the ventromedial nucleus (VMN) and medial preoptic area (POA) of the rat hypothalamus. $GABA_A$-mediated synaptic transmission within these regions has been shown to play a key role in the expression of adult female sexual behavior. We have characterised $GABA_A$-mediated responses in the VMN and POA of neonatal rats (PN0-PN20) by analysis of currents elicited by rapid perfusion of high concentrations of agonist in dissociated neurons and of spontaneous inhibitory postsynaptic currents (ipscs) recorded from neurons in intact slices. Responses were elicited by rapid application of 1 mM GABA for 1-2 ms from nucleated outside-out patches. Current decays were best fitted by three exponential components with time constants of ~ 10, 60, and several hundred ms. Spontaneous ipscs were recorded in acutely isolated slice preparations in the whole-cell configuration in the presence of 20 μM CPP and 10 μM CNQX. Spontaneous ipscs were blocked by 10 μM bicuculline. Time constants of ipsc decay were similar to those for currents elicited by rapid agonist perfusion. Statistical analyses suggest that currents recorded from VMN neurons of male versus female animals show significant differences in decay kinetics. Results will be discussed with regard to sex-dependent differences in $GABA_A$ receptor composition within these regions, as assessed by the sensitivity of currents to allosteric modulators and by differential expression of $GABA_A$ receptor subunit genes. Supported by NSF IBN-9319523 (LPH/ASC).

791.2

SEX DIFFERENCES IN GLUTAMIC ACID DECARBOXYLASE (GAD) mRNA CONTENT IN THE NEONATAL RAT BRAIN. M.M. McCarthy*, D.R. Grattan, A.M. Davis & M. Selmanoff, Department of Physiology, Univ. of Maryland, Baltimore, MD 21201

Although there are sex differences and steroid effects on the inhibitory transmitter GABA in the adult brain, a role for this neurotransmitter in the sexual differentiation of the neonatal brain has not been established. Glutamic acid decarboxylase (GAD) is the rate limiting enzyme in GABA synthesis and comes in two forms: GAD-65 and GAD-67. We have used a lysate version of the RNase protection assay to quantify mRNA for both forms of GAD in tissue micropunches from specific brain regions. Data are expressed as the ratio of GAD mRNA to that of a housekeeping gene, GAPDH. GAD mRNA was quantified in the arcuate nucleus (ARC), dorsal medial nucleus (DMN), medial preoptic area (mPOA), CA1 region of the hippocampus, amygdala (AMYG) and cingulate cortex (CTX) of individual male and female brains (n=8) during early (postnatal day 1, PN1), late (PN5) and post-critical period (PN15 and PN25) for sexual differentiation of the rat brain.

At PN1, GAD-65 and GAD-67 mRNA was almost twice as high in the DMN, and was significantly higher in CA1 region of males compared to females (ANOVA; $p<.05$). GAD-67 mRNA was also significantly higher in the ARC of PN1 males. No differences were detected in mPOA or CTX. At PN5, GAD-67 mRNA was significantly lower in the mPOA of males and GAD-65 was significantly higher in ARC. There were no other differences at PN5. At PN15, GAD-67 mRNA was significantly greater in the ARC of males and both forms of GAD mRNA were higher in the AMYG of males. There were no significant differences in other brain areas and no differences at PN25.

We conclude that varying levels of GAD expression during the critical period is involved in establishment of sexually dimorphic brain regions. Additional experiments will examine sex differences in adults and the impact of neonatal hormonal manipulations on GAD mRNA levels. *Supported by SRIS Grant to MMM from Univ. of MD, School of Medicine.*

791.3

EFFECT OF EXOGENOUS ESTRADIOL ON DEVELOPMENT OF THE DOPAMINE D1 RECEPTOR IN THE RAT. J.A. Williams* and S.S. Maswood. Dept. Biol. Southeastern Oklahoma State Univ., Durant OK 74701 and Dept. Biol. Texas Woman's Univ., Denton, TX 76204.

Endogenous testosterone is aromatized within brain cells to estrogen in males at critical periods during development. Females are protected from the effects of endogenous estrogens through the presence of alpha-feto protein. However, exogenous estrogens can function like testosterone and masculinize the female brain. In this study the effect of estradiol on development of the dopaminergic system was studied. Male and female F-344 rats were injected (s.c.) on post-natal day 3 with either 1 μg estradiol benzoate or sesame oil vehicle. Animals were sacrificed between post-natal days 26-35. Brains were dissected and frontal cortex, hippocampus, and striatum removed and frozen. The dopamine D1 receptor was assayed in these tissues using [^{3}H] dopamine as radioligand and the D1 agonist (±) SKF-81297 hydrobromide as the unlabeled competitor. Levels of [^{3}H] dopamine binding to the D1 receptor subtype were decreased in cortex and hippocampus of both males and females receiving neonatal estradiol, relative to male control animals. Conversely, D1 receptor binding was increased in the striatum of estradiol treated animals. These results suggest that estradiol affects development of the dopaminergic system in a region-specific manner.

791.4

IMMUNOHISTOCHEMISTRY AND IN SITU HYBRIDIZATION HISTOCHEMISTRY OF CORTICOTROPIN-RELEASING FACTOR(CRF)-CONTAINING NEURONS IN THE FETAL RAT BRAIN. T. Fujioka[1], I. Akimura, T. Watanabe, T. Shibasaki[2] and S. Nakamura* Dept. of Physilogy and [1]Dept. of Obstetrics and Gynecology, Yamaguchi Univ. Sch. of Med., Ube, Yamaguchi 755, and [2]Dept. of Medicine, Tokyo Women's Medical College, Tokyo, Japan.

Immunohistochemistry and in situ hybridization histochemistry were used to examine prenatal development of CRF-containing neurons in the rat brain. CRF mRNA was first expressed in the lateral hypothalamus and hypothalamic paraventricular nucleus at embryonic day (E)15 (E0: sperm-positive day) in parallel with CRF immunoreactivity. As development proceeds, CRF immunoreactivity of neurons in these areas became progressively enhanced, and the number of CRF-containing neurons increased. In addition, CRF-immunoreactive neurons appeared in other brain regions including the anterior hypothalamus, arcuate nucleus, amygdala, bed nucleus of stria terminalis and septal area. CRF-immunoreactive fibers in the median eminence were detected as early as E17. These findings indicate the expression of CRF-containing neurons in various brain regions during the prenatal period. We are further investigating the possibility of the coexistence of CRF with vasopressin in fetal brain neurons. (Supported by Grant in Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan).

791.5

TRANSIENT SEX DIMORPHISM IN THE NUMBER OF NADPH-DIAPHORASE-POSITIVE GANGLION CELLS OF THE RAT ADRENAL GLAND DURING POSTNATAL DEVELOPMENT. C. Cracco*, S. Biasiol, G. Filogamo and A. Vercelli. Department of Human Anatomy and Physiology, Torino Univ. Sch. Med., Italy.

The rat adrenal gland (AG) contains ganglion cells able to synthesize nitric oxide, a neurotransmitter/neuromodulator involved in the control of adrenal secretory activity and blood flow. In the present study we analysed the number and distribution of nitric oxide-producing adrenal neurons during postnatal development in the two sexes by means of NADPH-diaphorase (NADPH-d) histochemistry. Wistar albino rats were perfused at various postnatal ages and the AGs processed to visualize the NADPH-d. While in adulthood positive neurons are mostly located in the medulla, at birth they are equally distributed within cortex and subcapsular region in both sexes, the medulla being practically devoid of stained nerve cells. The adult distribution of the NADPH-d-positive neurons is reached by the end of the fourth week in both sexes. A transient sex-related difference in the number of NADPH-d-positive neurons per AG is found during the second postnatal week (P7: male 463 ± 35 vs. female 192 ± 6; P10: male 473 ± 54 vs. female 260 ± 61; P15: male 369 ± 43 vs. female 278 ± 62). If newborn male rats are flutamide-injected daily, the number of NADPH-d positive adrenal ganglion cells at P10 is strongly decreased to about half of the control values. On the contrary, if newborn female rats are injected at P0 with one dose of testosterone enantate, the number of positive neurons at P10 is nearly doubled.

In conclusion, the present data demonstrate a transient sex influence on the number of NADPH-d positive neurons in the rat AG, limited to the second postnatal week, and probably related to the perinatal difference in serum testosterone in the two sexes. (Supported by MURST grants to AV)

791.6

ESTROGEN INDUCES AN INCREASE IN SINGLE CELL EXPRESSION OF PROENKEPHALIN mRNA IN THE VL-VMN OF YOUNG AND ADULT FEMALE RATS. C. Gómez[1], A. Rodríguez [1], J.L. González [1], J.A. Inserni [2]*, B.S. McEwen[3] and A.C. Segarra[1]. University of Puerto Rico, Physiology Department[1] and Neurosurgery Department[2], San Juan, PR. 00936[1] and The Rockefeller University, NY, NY 10021[3].

Estrogenic regulation of proenkephalin expression in the ventrolateral-ventromedial nucleus of the hypothalamus (VL-VMN) was studied in two age groups of female rats: juveniles and young adults. The juveniles were injected with oil or estradiol benzoate (40 μg/kg) at days 21 and 22 and sacrificed at day 23. The adult group consisted of intact and gonadectomized rats that were injected with oil or estradiol benzoate (40μg/kg) at days 72 and 73 and were sacrificed at day 74. Sections of the VMN were studied by *in situ* hybridization histochemistry at the single cell level and quantified with the assistance of an image analysis system. Estrogen plasma levels were determined by radioimmunoasay. Estrogen increased the amount of proenkephalin mRNA expressed per cell in the VL-VMN of juvenile females. We also observed an increase in the amount of cells expressing low levels of proenkephalin mRNA in these rats. In the adult, ovariectomy reduced proenkephalin mRNA expression, estrogen restored mRNA levels to those of intact females.

791.7

ESTROGEN INDUCES AN INCREASE IN SINGLE CELL EXPRESSION OF PROENKEPHALIN mRNA IN THE VL-VMN OF YOUNG BUT NOT ADULT MALE RATS. Z. Rivera[1], M. Rivera [1], J.A. Angulo[2], and A.C. Segarra[1]* University of Puerto Rico, Physiology Department[1] San Juan, PR. 00936[1] and Biology Department, Hunter College, CUNY, NY, NY[2].

Estrogenic regulation of proenkephalin expression in the ventrolateral-ventromedial nucleus of the hypothalamus (VL-VMN) was studied in two age groups of male rats: juveniles and young adults. The juveniles were injected with oil or estradiol benzoate (40 μg/kg) at days 21 and 22 and sacrificed at day 23. The adult group consisted of intact and gonadectomized rats that were injected with oil or estradiol benzoate (40μg/kg) at days 72 and 73 and were sacrificed at day 74. Sections of the VMN were studied by *in situ* hybridization histochemistry at the single cell level and quantified with the assistance of an image analysis system. Estrogen increased the amount of proenkephalin mRNA expressed per cell in the VL-VMN of juvenile males. In the adult, neither orchidectomy nor estrogen treatment had an effect on proenkephalin expression in the VL-VMN. These results and those of Romano et al. (1990) and Hammer et al. (1993) indicate that estrogenic regulation of enkephalinergic expression in the VMN of male rats changes with age and duration of estrogen exposure.

791.8

WITHDRAWN

791.9

DIFFERENTIAL REGULATION OF CALCITONIN GENE-RELATED PEPTIDE AND SUBSTANCE P BY ESTROGEN IN RAT SENSORY NEURONS. S. Sarajari, J. Pickett, N. Taleghany and M.M. Oblinger* Dept. of Cell Biol. and Anat., Chicago Medical Sch., N. Chicago, IL

Calcitonin gene-related peptide (CGRP) and Substance P (SP) are co-expressed in a variety of neurons in the central as well as peripheral nervous system, including most small nociceptive neurons of the dorsal root ganglia (DRG). In regions of the nervous system that contain estrogen receptors (ER), such as pituitary and several hypothalamic regions, estrogen (E2) has been shown to augment CGRP levels and to downregulate SP levels. Recently, we have found that many of the small, but not the large-sized, DRG neurons express high levels of ER. Since many of these same neurons also express SP and CGRP, it was of interest to determine how hormonal status affects peptide expression in the DRG. Female Sprague Dawley rats were ovariectomized and either treated continuously with exogenous E2 via a subcutaneous implant of a silastic capsule filled with 17β-estradiol for 21 days (+E group) or were left untreated for that same period (ovx group). Lumbar and sacral DRGs were harvested and immunostained using a variety of polyclonal and monoclonal antibodies to CGRP, SP or ER. Results showed robust CGRP immunoreactivity (IR) in most small-sized DRG neurons, as well as in some medium to large-sized neurons; SP -IR was largely restricted to the small neurons. CGRP - IR in the DRG was markedly enhanced in the +E group relative to the ovx controls, but SP-IR appeared to be less robust in the +E group. This differential regulation of peptide expression in the DRG is consistent with findings in other ER containing neuronal populations. Studies have been recently initiated to explore the functional consequences of peptide modulation in the DRG by E2. Preliminary findings suggest that temperature/pain thresholds (tail flick response) may be influenced by gonadal steroid status.

791.10

LOCALIZATION OF NURR 1- AND NGFIB mRNA IN THE DEVELOPING AND ADULT MOUSE AND RAT CNS. R.H. Zetterström*, R. Williams, E. Lindqvist, T. Perlmann and L. Olson. Departments of Neuroscience and Cell & Molecular Biology, Karolinska Institute, S-171 77 Stockholm, Sweden.

Nurr 1 (nur-related 1) and NGFIb (nerve growth factor-inducible) are two closely related orphan receptors belonging to the steroid hormone receptor superfamily, which are both encoded by immediate early genes. The function of these two proteins is unclear, but in a recently published study the two orphan receptors have been shown to be able to modify retinoid signaling. Other recent studies have indicated that they might be involved in apoptosis. In order to further elucidate the role of these proteins we have applied in situ hybridization histochemistry to localize the possible presence of nurr 1 and NGFIb mRNA in the developing and adult mouse and rat CNS. Expression of nurr 1 mRNA was found in several regions during early development; this expression persisted throughout the pre- and postnatal period and was also found in many areas in the adult CNS. Nurr 1 mRNA was found in the olfactory bulb, several parts of the cortex, in the hippocampal formation, in the ventral tegmental area and in the substantia nigra. In contrast to nurr 1, NGFIb mRNA expression was not found in the prenatal CNS. NGFIb mRNA was first detected in newborn animals. Morover, the transcript was almost exclusively found in small groups of cells in the basal ganglia. Postnatally the NGFIb expression increased and in the adult brain NGFIb mRNA was found in many different regions, including the olfactory bulb, striatum, hippocampal formation and cortex. The interesting pattern of distribution of these two receptors imply important functions in the CNS both during development and in adulthood.

791.11

NGF-RECEPTOR IMMUNOCYTOCHEMISTRY PROVIDES EVIDENCE FOR INCREASED NEURONAL PROCESSES IN BASAL FOREBRAIN CHOLINERGIC NEURONS OF RATS RECOVERING FROM EARLY HYPOTHYROID BRAIN RETARDATION. A. Farahvar* and E. Meisami (Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801).

Plasma thyroxine levels were suppressed in rat pups from birth by addition of PTU (propylthiouracil) in drinking water (1g/L). This neonatally induced hypothyroidism results in profound retardation of neuronal growth in brain regions. Recovery from this condition was initiated by withdrawal of PTU at 25 days of age. Animals recovering from hypothyroid show markedly increased growth rates in body and brain regions including hippocampus and dentate gyrus. Expression of low affinity p^{75} NGF-receptor (NGF-R) is thought to be largely confined to cholinergic neurons of the basal forebrain (BF) which project extensively to forebrain areas. We have shown that compared to 25-day control rats, there is a significant elevation in NGF-R levels in BF neurons of hypothyroid rats. BF neurons were stained immunocytochemically using a monoclonal antibody to NGF-R (192-IgG, Boehringer-Mannheim). Three weeks after PTU withdrawal, NGF-R immunoreactive neurons of BF in recovering rats showed markedly increased neuritic outgrowth and branching as compared to age-matched controls. This neuritic sprouting was most evident in neurons of magnocellular BF nuclei. The above results suggest regulation of NGF-R levels in BF cholinergic system by thyroid hormones may underlie the markedly increased growth rates in rat brains recovering from hypothyroid retardation.

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791.12

NEUROTROPHIC FACTOR EXPRESSION IN THE ANDROGEN-SENSITIVE BULBOCAVERNOSUS MUSCLE. J. Xu and N.G. Forger*. Program in Molecular and Cellular Biology and Department of Psychology, Univ. of Massachusetts, Amherst, MA 01003.

Motoneurons of the spinal nucleus of the bulbocavernosus (SNB) of rats innervate striated perineal muscles including the bulbocavernosus (BC) and levator ani (LA). The survival of SNB motoneurons and their target muscles depends on androgen during perinatal development. As a result, the muscles and motoneurons persist in males, but degenerate in females. It has recently been demonstrated that ciliary neurotrophic factor (CNTF), can mimic some of the effects of early androgen in this system. CNTF prevents the death of SNB motoneurons and BC muscles that would normally occur in female rat pups. These observations raised the possibility that neurotrophic molecules might mediate some effects of androgen on the developing SNB system. We have used Northern blot analysis to begin to examine expression of neurotrophic factors and their receptors in the SNB system. The BC muscle of newborn male rats exhibited little or no expression of CNTF, but abundant expression of the α-component of the CNTF receptor (CNTFRα) and of TrkB, the high affinity receptor for the neurotrophins, brain-derived neurotrophic factor (BDNF) and NT-4. Expression of both CNTFRα and TrkB was very low in adult BC, indicating developmental regulation of these messages. To determine whether the expression of neurotrophic factors or their receptors might be androgen regulated, rats were treated with testosterone propionate or hydroxyflutamide, a potent antiandrogen, from embryonic day 20 (E20) through postnatal day 4 (P4). Expression of CNTF, BDNF, NT-4, CNTFRα and TrkB was then determined by Northern analysis on E20, P1 and P5 in the BC, LA, "thigh" muscle and lumbosacral spinal cord. (Supported by NIH grant HD33044-01 and the Whitehall Foundation.)

792.1

EFFECTS OF PRENATAL PROTEIN DEPRIVATION ON CORTICOSTERONE LEVELS IN ADULT RATS. P.D. Butler[1,2]*, D.A. Klugewicz[1,2], D. Ciplet[1], and J. Rotrosen[1,2]. Psychiatry Service, NYDVAMC, NY, NY 10010[1] & Dept. of Psychiatry, NYU Medical Center, NY, NY 10016[2].

Prenatal protein deprivation in rats has been used to probe long-term consequences of early nutritional deprivation. Altered hippocampal morphology and electrophysiological functioning, as well as increased 5-HT and 5-HIAA levels in a number of brain areas have been found. Corticosterone (CORT) levels are known to be regulated by brain 5-HT as well as glucocorticoid receptors in hippocampus. As part of a broader study in which we examined the behavioral, morphological and neurochemical effects of prenatal protein deprivation, we examined whether there would be long lasting changes in basal levels of CORT or CORT response to stress. Five weeks prior to breeding, SD rats were fed either a 6% or a 25% casein diet (Harlan Teklad, Madison, WI). All pups were cross-fostered to 25% dams who had given birth within the same 24 hour period, so that the experimental group was designated 6/25 and the control group 25/25. Pups were weaned at PND 25 and maintained on the 25% casein diet for the duration of the study. At PND 76-78 tail blood was obtained from a small group of rats at baseline, following 30 and 60 min of restraint, and after one hour recovery. CORT levels were measured by RIA (ICN Biomedicals). Both 6/25 (n=7) and 25/25 (n=7) animals showed a robust response to stress as seen by at least a 4-fold increase in CORT levels at 30 and 60 min. However, sample size was not sufficient to determine if the 2 groups differed in their response to stress. At PND 85, rats were sacrificed by decapitation and trunk blood collected and analyzed for CORT levels. 6/25 males (n=9) had significantly higher basal CORT levels (mean ± SEM; 215.3 ± 61.5 ng/ml) than 25/25 males (n=14; 70.2 ± 15.6 ng/ml; p=.048). 6/25 females (n=10) had a trend toward increased CORT (313.5 ± 50.5 ng/ml) compared to 25/25 females (n=14; 206.2 ± 44.5 ng/ml; p=.127). Thus, there appears to be a long lasting increase in basal levels of CORT in prenatally protein deprived rats.

792.2

EFFECTS OF CORN-FEEDING AND PROTEIN RESTRICTION ON DEVELOPMENT OF GABA-ERGIC CELLS OF THE CEREBRAL CORTEX IN RATS. HISTOLOGICAL AND IMMUNOCYTOCHEMICAL STUDIES. S.Orozco- Suàrez , A. Feria-Velasco* and A. Del Angel- Meza. Unidad de Invest. Med. en Enf. Neurológicas, H.Especialidades, CMN, S-XXI, IMSS, Mèxico, D.F. and Div. Patol. Exptl. Centro de Invest. Biomédica de Occidente, IMSS, Guadalajara, Jal. Mèxico.

Nutritional deficiency in early life affects the development of the central nervous system; there is a permanent deficit in cell number, and cell maturation is retarded in terms of axonal growth, myelination and enzymatic development. These changes are accompanied by a delay in the appearance of innate behavioral patterns, and which seem to be permanently impaired. The effects of undernutrition in early life have been recently studied in our laboratory in the following aspects: brain development, postnatal cell formation and development of GABAergic innervation of the sensorymotor cerebral cortex. Normal adult pregnant Wistar rats were fed for 6 weeks with 1) Normal diet (commercial diet for rodents, 23 % protein), 2)Hypoproteic (8 % protein) diet on a chow- purina base and 3) corn-based diet (with 8 % protein; low tryptophan and lysine diet) .The histologic studies were performed in the pups 1, 7, 14, 21, 30 and 60 days after birth. GABAergic elements were analyzed by GABA-immunocytochemistry. Radial distribution of cells and laminar numerical densities were calculated at each stage of development by photomicrographs systematically obtained from the total cortex thickness. Results of these studies showed that cortical thickness, total cellularity and the GABAergic cells were significantly reduced in the corn-fed group. The statistical significance of the difference was lower compared with the 8 % protein diet group. Results suggest that omission of essential nutrients from maternal diet has a greater inhibitory effect on postnatal brain development than that produced only by protein restriction.

792.3

EFFECTS OF PROTEIN MALNUTRITION ON HIPPOCAMPAL GABAergic CELLS. A. González-Maciel, M.A. Morales*, S. Díaz-Cintra. UISI-INP. SS. 14410, Depto. Biología Celular, IIB y Centro de Neurobiología, UNAM, México, D.F. 04510.

The effects of protein malnutrition on GABAergic cell density (GAD-positive cells) was studied in the fascia dentata and hippocampal formation of 30 and 90 days old rats. Five weeks prior to mating female rats were fed a 6% casein diet while control rats were fed a 25% casein diet. Malnutrition was established prenatally (6/25), postnatally (25/6), or both (6/6). At 30 days of age, the 6/6 malnourished group showed both a 31% significant ($p<0.0008$) reduction in the hippocampus, and a 39% significant ($p<0.0001$) decrease in the dentate gyrus GABAergic cell density. Conversely, at the same age, the 6/25 group displayed a 27% significant ($p<0.01$) increase in the fascia dentata GABAergic cell density. No significant differences were found at 90 days of age in hippocampal formation.

These data suggest that malnutrition affects the hippocampal formation morphology differentially. The increase in GABAergic cell density found at 30 days old in the 6/25 group indicates a delay in the maturation of hippocampal formation inhibitory circuitry. A severe decrease in GABAergic cell density was found in the 6/6 group in the fascia dentata and hippocampal formation. In the same group, however, a recovery was found in both structures in 90 days old rats. **Supported by DGAPA IN-204093.**

792.4

EEG POWER SPECTRA ANALYSIS BEFORE AND AFTER REM SLEEP DEPRIVATION IN NORMAL AND MALNOURISHED RATS OF 60 DAYS. L. Cintra*, P. Durán, A. Galván and S. Díaz-Cintra. Centro de Neurobiología, UNAM, A.P. 70228, México, D.F. 04510

It has been demonstrated that chronic protein malnutrition delays the maturation of the mechanisms that regulates the vigilance states. This study was designed to evaluate the effects of protein malnutrition induced by a 6% casein diet in rats, instituted 5 weeks before mating and continued during gestation and into postnatal life to their offspring, after REM-sleep deprivation in a "conflict experiment", using an inverted dark-light cycle (12D/12L). A base-line (BL) recording day at 60 days of age was followed by one day of REM-sleep deprivation by the platform technique in control and malnourished rats, followed by three recovery days. We found using EEG power spectra analysis, that the EEG relative power density of theta frequency band (4-7 Hz) was significantly increased in both light and dark phases of BL day one, during the first hour of dark phase of recovery day one and at initial hour of light phase of recovery day 2, and during first and last hour of light phase of day 3. Theta frequency band (7-12 Hz) was significantly reduced during first hour of dark phase of BL. This results suggest that malnutrition affects the mechanisms in central nervous system that regulates the theta rhythm. **Supported by DGAPA IN-208494**

792.5

PRENATAL PROTEIN MALNUTRITION ALTERS HIPPOCAMPAL LTP MEASURES AT ALL STAGES OF DEVELOPMENT. P.J. Morgane,* J.D. Bronzino, R.J. Austin-LaFrance and J.R. Galler, Center for Behavioral Development and Mental Retardation, Boston Univ. School of Medicine, Boston, MA 02118 USA.

The ability of prenatally malnourished rats to establish and maintain long-term potentiation (LTP) of the perforant path/dentate granule cell synapse was examined in freely-moving animals at 15, 30 and 90 days of age. Measures of the population EPSP slope and population spike amplitude (PSA) were calculated from dentate field potentials obtained prior to and following tetanization of the perforant path. Significant enhancement of both measures was obtained from all animals of both the diet and control groups at 15 days of age. The magnitude of enhancement obtained from the malnourished group was significantly less than that of age-matched, well-nourished controls. At 30 days of age, PSA measures obtained from approximately 50% of malnourished animals showed no significant enhancement, while measures obtained from the remaining 50% did not differ from controls. EPSP slope measures for this age group followed the same pattern. At 90 days of age, PSA measures also showed a bimodal distribution, with 50% of malnourished animals initially showing a decline in PSA measures, while the remaining 50% did not differ from controls. These results indicate that gestational protein malnutrition has an enduring impact on hippocampal neuroplasticity. **Supported by NIH/NICHD Grant # HD22539**

792.6

PRENATAL EXPOSURE TO MALNUTRITION BUT NOT COCAINE IMPAIRS ACQUISITION IN THE RADIAL ARM MAZE. J.S. Shumsky*, P.L. Shultz, J.R. Galler, and J. Tonkiss. Center for Behavioral Development & Mental Retardation, Boston University School of Medicine, 80 E. Concord St., Boston, MA 02118.

Maternal cocaine abuse is often associated with malnutrition. To explore the separate and combined effects of these two insults, the acquisition of a spatial task was examined in the adult male offspring of female rats exposed to protein malnutrition (6% casein diet) and/or cocaine (30 mg/kg) for 5 wks prior to mating and during pregnancy. Control rats were fed a 25% casein or chow diet and saline-injections were performed as drug-injection controls for all diet groups. Rats were tested using the 8-arm radial maze with 4 arms baited and were required to collect all 4 food pellets within 5 min to complete a trial. Subjects were tested for 1 trial/day until they each acquired the task. Acquisition of this task was completed when the rats attained the stringent performance criterion of obtaining 3 out of the 4 food pellets within their first 4 arm entries (while still completing the trial) over 3 consecutive days. The results showed a clear dissociation between the effects of prenatal cocaine and prenatal malnutrition. There were no significant effects due to prenatal cocaine on any measure in this procedure and there were no additive effects with prenatal malnutrition. However, prenatally malnourished rats showed impairment in two measures of acquisition: number of trials to criterion performance and task efficiency (correct arm entries/total arm entries x 100) when compared to controls. No differences were observed in the number of working or reference memory errors, suggesting that the insult produced a general impact on the acquisitional process. These results suggest that prenatal malnutrition, but not cocaine, can impair the acquisition of a radial arm maze task, when a complex procedure with a stringent performance criteria is employed. Supported by NIH grant DA 07934.

792.7

BEHAVIORAL EFFECTS OF PRENATAL PROTEIN DEPRIVATION IN PRE-WEANLING AND ADULT RATS. D.A. Klugewicz[1,2]*, P.D. Butler[1,2], D. Ciplet[1], and J.Rotrosen[1,2]. Psychiatry Service, NYDVAMC, NY, NY 10010[1] & Dept. of Psychiatry, NYU Medical Center, NY, NY 10016[2].

Recent evidence indicates that prenatal nutritional deprivation may be one early environmental exposure that increases the risk of schizophrenia. In line with a neurodevelopmental model of SCZ, we investigated the effects of prenatal protein deficiency on growth rate, developmental milestones, and dopamine mediated behaviors in pre- and post-pubertal rats using dopamine agonists and antagonists. Five weeks prior to breeding, virgin female SD rats were fed either a 6% or a 25% casein diet (Harlan Teklad, Madison, WI). All pups were cross-fostered to 25% dams who had given birth within the same 24 hour period so that the experimental group was designated 6/25 and the control group 25/25. Pups were weaned at PND 25, and maintained on the 25% casein diet for the duration of the study. Weights were measured at birth, and approximately every third day thereafter. As expected, weights of 6/25 animals did not differ from 25/25 animals at birth or anytime thereafter. From PND 1-21, pups were assessed for the attainment of developmental milestones and reflexes. Surprisingly, 6/25 animals attained several developmental milestones (i.e. righting reflex, negative geotaxis, acoustic startle, ear unfolding and incisor eruption) before 25/25 animals. At PND 35 and PND 56, stereotypy was measured in rats receiving apomorphine (0.75 mg/kg, SC). At both ages, 6/25 females (n=9), but not males, had a trend towards increased stereotypy levels compared to 25/25 females (n=15), which reached significance only at PND 56. However, d-amphetamine (1.5 mg/kg, IP) induced locomotion and haloperidol (1.0 mg/kg, IP) induced catalepsy did not differ between 6/25 and 25/25 rats at either age, though 6/25 females (n=8) showed a trend toward increased amphetamine induced locomotion at PND 56. Our results suggest that prenatal protein deprivation alters the attainment of developmental milestones, and selectively increases some dopamine-mediated behaviors in post-pubertal, but not pre-pubertal rats.

792.8

TRANSGENERATIONAL EFFECTS OF A PRENATAL TRYPTOPHAN (TRP) DIET. Moto Sakuma*, Moto R.R.C.Co., 4434 Grayton, Detroit, Mi 48224.

The manipulation of a single amino acid in pregnant laboratory rats may have an effect on their progeny. During the third trimester,five Sprague-Dawley pregnant rats were given a 3%TRP diet, and another five received a 0.3%TRP diet(control). In the preweaning period of the first generation(F1) whose mothers (F0) were given the 3%TRP diet(n=35), significant delayed growth was noted in comparison to the growth noted in the control(n=23). In the postweaning to adult period, all 35 F1 rats of F0's given the 3%TRP diet displayed significant slowness in open-field behaviors, sensitive-emotionality and heavier body weights when scored at 3,6,10 and 15 weeks of ages. Heavier brain weights were noted both birth and weaning time in these F1 rats as compared to the control. As a result of sibling mating, 59 second generation(F2) rats, whose grandmothers(F0) were given 3%TRP diet, revealed longer periods of corner crouching, higher circling,longer latency in rearing,fewer rearing counts, less defecation and heavier body weights than that of 21 control F2 rats in their postweaning to adult period. The hypoactivity and emotionality of these F2 rats were noted to be more pronounced that those observed in the F1 rats. Phenotypic evidence may be interpreted as having a transgenerational effect as the result of a prenatal TRP diet. The OPRR guidline will be followed for care.

792.9

CHRONIC PLACENTAL INSUFFICIENCY IN FETAL GUINEA PIGS AFFECTS NEUROCHEMICAL AND NEUROGLIAL DEVELOPMENT BUT NOT NEURONAL NUMBERS IN THE BRAINSTEM. S.M. Rees* and M.Tolcos. Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Victoria, 3052, Australia.

It is now widely believed that infants dying of sudden infant death syndrome might have a subtle underlying immaturity of the brainstem perhaps resulting from development in a compromised intrauterine environment. In this study we have examined the effects of chronic placental insufficiency induced by unilateral ligation of the maternal artery at mid-gestation (term=66-68 days) in guinea pigs (n=29). The prenatal development of nuclei in the brainstem involved with cardiorespiratory control and swallowing was analysed using stereological procedures and immunohistochemistry. A method was devised to enable the techniques to be performed on alternate frozen sections. The total number of neurons, area of neuronal somata and volume of the hypoglossal nucleus, showed no significant difference between control and compromised fetuses. There was a proliferation of astrocytes, as determined by immunoreactivity to glial fibrillary acidic protein, in the dorsal motor nucleus of the vagus, nucleus tractus solitarius (NTS) and around blood vessels throughout the brainstem. Immunohistochemical analysis of neurochemicals in the brainstem of compromised fetuses compared to controls, revealed a decrease in substance P (SP) immunoreactivity in the spinal trigeminal tract; a significant ($p < 0.05$) increase of 33% in the number of SP-positive neurons in the NTS and an increase in met-enkephalin (ME) immunoreactive fibres in the hypoglossal nucleus. These results show that although chronic intrauterine deprivation does not alter neurogenesis, at least in the hypoglossal nucleus, there is a proliferation of astrocytes and the expression of neurotransmitters/neuromodulators is affected in nuclei involved with cardiorespiratory control and swallowing.

792.10

IMPAIRED SOCIAL RECOGNITION MEMORY IN RATS OF BOTH SEXES EXPOSED TO ALCOHOL DURING THE BRAIN GROWTH SPURT. T.D. Tran and S.J. Kelly*, Dept. Psychology, Univ. of South Carolina, Columbia, SC 29208.

A test of social recognition memory in rats involves exposing them to a juvenile conspecific for investigation during one session and after a delay, exposing them to the same juvenile for another session. A reduction in investigation time during the second session suggests memory of the juvenile. This study addressed whether alcohol exposure affects adult rats' social recognition memory. From postnatal day 2 through 10, rats in the alcohol group were intubated with 3 g/kg/day of ethanol in milk formula. Two hours later, they were given milk formula alone. Mean blood alcohol concentrations reached 255.5 ± 31.1 mg/dl. Two control groups were either intubated and not exposed to alcohol or not exposed to both the intubation and alcohol. Rats were weaned at 21 days and group-housed. No differences in body weight were found among the groups. Behavioral testing of adult rats occurred over three days. Prior to the first session of each test day, rats were placed in a cage with fresh bedding for 15 min. On day 1, a juvenile (21-30 days old) was placed in the rat's cage for 5 min and investigation time was recorded using an S&K event recorder. After a 90 min inter-exposure interval (IEI), the rat was reexposed to the same juvenile for 5 min and investigation time was again recorded. On days 2 and 3, IEIs of 30 min were used, and either the same juvenile (Day 2) or a different juvenile (Day 3) was used in the second session. Testing occurred during the light portion of the day, and females were only tested during diestrus; daily vaginal smears verified the stage of estrus. Across all groups, males spent more time investigating the juveniles in the first session than females; no differences were found among groups. Ratios of second session to first session investigation times were examined. Scores of 1.0 indicated no memory retention. There were no differences in the ratios between the sexes and when scores were collapsed across sex, alcohol-exposed rats showed marked impairment in recognizing the same juvenile in the second session after a 90 min IEI, compared to the two control groups which did not differ. Results suggest that early postnatal alcohol exposure impairs rats' social memories. (Supported by a Carolina Venture Fund Grant to S.J.K.)

792.11

ETHANOL ALTERS THE CONTENT OF PITUITARY AND BRAIN B-ENDORPHIN ON THE 20TH DAY OF FETAL LIFE IN THE RAT. H-L Li and C Gianoulakis* Douglas Hospital Research Centre Verdun, Quebec Canada H4H 1R3

At the time of birth and early postnatal life the total content and concentration of B-endorphin in the pituitary gland was lower, while the concentration of hypothalamic B-endorphin was higher in the fetal ethanol than control offspring. In the present studies the content of B-endorphin peptides in the fetal pituitary and distinct regions of the fetal brain were measured on the 20th day of gestation when the offspring are not experiencing the effects of ethanol withdrawal. Rats were fed during gestation (a) with a liquid ethanol diet ad libitum; (b) were pair-fed to the ethanol fed animals with an isocaloric liquid diet; and (c) were fed with laboratory chow and water ad libitum. On the 20th day of gestation the fetal pituitary and distinct regions of the fetal brain (accumbens, frontal cortex, septum, arcuate nucleus, amygdala, hippocampus, ventral tegmental area, and central gray matter) were dissected and extracted in 0.1 normal HCL for estimation of the content of B-endorphin peptides. Results indicated a lower content of B-endorphin in the pituitary gland, and slightly higher content of B-endorphin in the brain regions, of the ethanol exposed offspring. These ethanol induced changes in the content of B-endorphin may alter the process of neurogenesis in the fetal ethanol offspring . Supported by a grant from the NIAAA.

792.12

PERINATAL METHADONE EXPOSURE AND RAT PUP DEVELOPMENT. P.M. Kunko*, J.A. Smith, M.J. Wallace, D.T. Otey, J.R. Maher and S.E. Robinson. Dept. of Pharmacology and Toxicology, Med. College of Virginia, Richmond, VA 23298.

This study examined pup development and behavior following pre- and postnatal methadone (M) exposure, and postnatal M withdrawal. Sprague-Dawley CD rat pups, derived from dams treated with water (W) or M (9mg/kg/day) via osmotic minipumps, were cross-fostered on postnatal day 1 (PD1) to W- or M-treated dams, producing four prenatal/postnatal treatment groups: W/W, W/M, M/W, M/M (pumps implanted gestational day 7, with replacement pumps on PD10). Pups were weighed daily and examined for the appearance of developmental milestones. One group of litters (pups and dams) was challenged with naloxone (1mg/kg, s.c.) to test for physical dependence on PD19. Pups from remaining litters were examined for locomotor activity in an open field on PD21. M reduced food and water intake in the dams, but not body weight. M-treated dams had smaller litters and smaller pups and, in general, deficits persisted through PD10. Postnatal M decreased pup weight from PD7-21. Postnatal M exposure appeared to enhance some development, decreasing latency for the righting reflex in W/M pups and decreasing latency for the negative geotaxic response in W/M and M/M pups. M-treated dams and W/M and M/M pups exhibited signs of withdrawal upon naloxone challenge. M/W and M/M pups exhibited less spontaneous locomotion in the open field during the initial 5-min observation. The results suggest both transient and lasting effects of prenatal M exposure and withdrawal, and deficits associated with postnatal exposure. [Supported by 1P50 DA05274]

792.13

PERINATAL OPIOID EXPOSURE AFFECTS CHOLINERGIC DEVELOPMENT IN THE RAT. S. E. Robinson,* Q. Mo, M. J. Wallace, J. R. Maher, D. T. Otey, and P. M. Kunko. Department of Pharmacology & Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613.

The present study was performed to determine whether perinatal exposure to full (methadone, M) or partial (buprenorphine, B) μ-opioid agonists delays the development of striatal cholinergic neurons. On day 7 of pregnancy, Sprague-Dawley CD rats were anesthetized with methoxyflurane and implanted s.c. with osmotic minipumps filled with M (9 mg/kg/day), B (1.5 mg/kg/day) or sterile water (W). Maternal weight, food and water intake were monitored throughout pregnancy. Within 24 h of birth, litters were culled to 10 and fostered to dams implanted with minipumps containing M, B or W, so that W/W, M/W, W/M, M/M, B/W, W/B, and B/B prenatal/postnatal exposure groups were obtained. New pumps were implanted in dams on postnatal day (PD) 10. Choline acetyltransferase (ChAT) mRNA was measured by Northern blot analysis in the striata of 10- or 22-day-old male pups, using an α-^{32}P dCTP-labelled 300 bp ChAT cDNA probe. The ratio of ChAT mRNA to 28S RNA was significantly decreased in 10-day-old pups exposed to either M or B perinatally. By PD 22, only pups with continued postnatal exposure to M had reduced ChAT mRNA. Therefore, M and B reduce expression of mRNA for ChAT, which may reflect a delay in the development of striatal cholinergic neurons. However, expression of ChAT mRNA returns to control values by PD 22 in the B-exposure group and in pups after exposure to M is stopped. [This research was supported in part by NIDA grant 1P50 DA05274].

792.14

SUBSTANCE ABUSE DURING PREGNANCY IN A HISPANIC POPULATION: PREVALENCE AND EFFECTS ON PROGENY. N.E. Del Valle[1*], Z. Rivera[1], J.A. Capriles[2], M.P. Casado[2] and A.C. Segarra[1]. University of Puerto Rico, Physiology Department[1] and Municipal Hospital, Department of Pediatrics[2], Medical Center, San Juan, Puerto Rico 00936.

A sample of newborn children in the San Juan area Municipal Hospital was taken for a prospective longitudinal study. Each week, ten neonates, 5 males and 5 females, of less than 24 hrs were randomly selected to participate in the study. Urine samples of the newborn were collected and assessed by radioimmunoassay for cocaine and nicotine. Birth parameters such as birth weight, length, head circumference and fontanel size were recorded. In our sample, no neonates tested positives for morphine, 6% tested positive for cocaine and 14% tested positive for nicotine. A decrease in birth weight and length was observed in neonates whose urine tested positive for cocaine but not in nicotine-positive neonates. No effect was observed in head circumference nor in fontanel size in these nicotine and cocaine positive neonates. We also observed a sex difference in birth weight and length, males being larger and weighing more than females. No sex difference was observed in head circumference nor in fontanel size. We are currently in the process of establishing nicotine and cocaine urine cut-off values in neonates.

792.15

THE EFFECTS OF PRENATAL NICOTINE EXPOSURE ON DEVELOPMENTAL CHANGES IN BRAIN BIOGENIC AMINE CONTENTS. K. Muneoka*, K. Kamei, S. Muraoka, R. Tomiyoshi, T. ogawa and M. Takigawa. Dept. of Neuropsychi., Faculty of Med., Kagoshima Univ., Kagoshima and Safety Research Department, Teikoku Hormone Mfg. Co., Ltd., Kawasaki-shi, Japan.

We have reported that prenatal stressful treatments such as injections, or glucocorticoids administration affected the development of serotonergic neuronal system in rat brain. On the other hand, prenatal nicotine exposure via maternal injections is a treatment inducing hypoxia and ischemia to fetus, that is mimic to the effect of maternal tobacco use. Some studies indicate that effects of nicotine itself can be discriminated from overall effects of nicotine exposure by using infusions. In the present study, we investigated effects of maternal nicotine injections(inj.) or infusions(inf.) on monoaminergic neuronal developments including serotonergic system. Pregnant SD rats were exposed to nicotine via injections twice a day (6mg/kg/day) or infusions by implanted osmotic minipumps (flow rate; 6mg/kg/day) from gestational day (GD) 4 to GD 20. In the midbrain+pons-medulla (M+P-M) and the forebrain of rat pups on postnatal day (PD) 7 and PD 15, NE, DA, DOPAC, HVA, 5HT and 5HIAA contents were measured by HPLC with ECD. Data were analyzed by two-way ANOVA (factors of treatment (inj. or inf.) and nicotine). Main significant changes are summarized below. In M+P-M, effects of inj. treatments were increases in NE, DOPAC, HVA, 5HT and 5HIAA. Effects of nicotine were decrease in HVA and increase in 5HT. In forebrain, effects of inj. were increases in NE, DOPAC, HVA and 5HIAA, and decreases in DA and 5HT. Effects of nicotine were increase in DA and decrease in DOPAC. In addition, these changes in M+P-M and forebrain were dominant in PD7 and PD15 respectively. Our results indicated that nicotine exposure affected not only dopaminergic system but serotonergic system while inj. treatment was likely to affect various aminergic systems.

792.16

SEX DIFFERENCES IN BODY WEIGHT EFFECTS OF PRENATAL NICOTINE IN RATS S. M. Nespor, E. J. Popke, M. A. Rahman, Y. Tizabi,and N. E. Grunberg* Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799; and Dept. of Pharmacology, Coll. of Med., Howard University, Washington, DC 20059

Maternal cigarette smoking during pregnancy results in decreased infant birthweight. These effects are consistent with the inverse relationship between cigarette smoking and body weight and between chronic nicotine administration and body weight. The effects of smoking on body weight in adults result from effects of nicotine on energy intake and energy expenditure and may involve effects of nicotine on plasma and hypothalamic insulin levels. The present experiment examined effects of prenatal nicotine exposure on the body weights and blood and hypothalamic insulin levels of Sprague-Dawley rat pups during the first five weeks post partum. Twenty-four pregnant dams received nicotine (6 mg/kg/day) or saline, administered by osmotic minipump, during days 4-22 of the 22-day gestation period. Body weights for the 118 male offspring and 104 female offspring were measured 18 days, 24 days, 30 days, and 34 days post partum. Blood and hypothalami from these offspring were assayed by radioimmunoassay for insulin levels. Nicotine significantly and consistently reduced the body weights and the plasma and hypothalamic insulin levels in female offspring. Nicotine slightly reduced the body weights and the plasma and hypothalamic insulin levels in male offspring. In addition, for female and males, the body weights correlated positively with the plasma and hypothalamic insulin levels ($p<0.01$). These results are consistent with previous reports that nicotine affects body weight and insulin levels and that females are more sensitive to these effects of nicotine..

792.17

IN UTERO EXPOSURE TO PAROXETINE LEADS TO ALTERED PHYSICAL AND MOTOR DEVELOPMENT. J. H. Patton*, C. H. Rayer, J. L. Langdoc. Neuroscience Program, Baylor University, Waco, TX, 76798-7334.

Previous research in our laboratory has demonstrated that in utero exposure to fluoxetine can cause morphological and behavioral teratogenic effects (Stanford & Patton, *Pharm. Biochem. & Beh.*, 1993, 45, 959-962; Patton, Langdoc, & Rayer, Poster Presented at SWPA, 1995). To test the generalizability of these effects, we exposed dams to approximately seven times the maximal human dose of paroxetine on a mg/kg basis (4.66 mg/kg) via oral gavage beginning on day seven of mating and ending the day of birth. Control animals received distilled water in similar volume by gavage during the same time period. On day three, all litters were culled to eight members, and individual pups were weighed, measured, and toe clipped for identification. Behavioral assessment began on day five. Paroxetine exposed litters had an increased number of stillbirths and neonatal deaths (Stillbirths: $t = -2.26$, $p = 0.037$, $df = 17$; Neonatal deaths $t = -2.49$; $p = 0.023$, $df = 17$). In utero paroxetine exposure had no effect on weight and length, nor upper and lower incisor protrusion time; paroxetine exposure did, however, result in earlier eye openings ($F_{1,125} = 23.043$, $p < 0.001$). Although paroxetine pups performed similarly to controls on surface righting, paroxetine exposed pups performed faster on tests of negative geotaxis (Latency: $F_{1,122} = 4.33$, $p = 0.039$; Trials to Criterion: $F_{1,126} = 4.516$, $p = 0.036$). The paroxetine pups also required fewer trials to reach criterion on cliff avoidance ($F_{1,126} = 3.218$, $p = 0.050$). Combined with our previous work with fluoxetine, results of this study suggest caution in the administration of SSRIs during pregnancy.

793.1

IDENTIFICATION OF CORTICOSTERONE-RESPONSIVE GENES INVOLVED IN HIPPOCAMPAL DEGENERATION. E. Vreugdenhil,* J. de Jong, J. S. Busscher and E.R. de Kloet. Leiden/Amsterdam Center for Drug Research, Division of Medical Pharmacology, PO box 9503, 2300 RA Leiden.

Fluctuating levels of adrenal corticoids play a pivotal role in the viability of hippocampal neurons. In animal experiments, removal as well as excess corticosteroids may result in the neurodegeneration of hippocampal neuronal circuits and consequently result in deficits in cognition and in adaptation to stress.

To investigate the molecular mechanisms underlying corticosteroid-mediated neurodegeneration in the hippocampus we have applied the differential display technique to compare the hippocampal expression profile of four groups of animals. We have used ADX rats and compared these with sham-operated animals. Furthermore, as glutamate is an important additional factor in hippocampal neurodegeneration, kainic acid was administrated to ADX rats and to sham operated rats. The display of approximately 5000 gene products results in the identification of two differentially expressed products between the ADX and sham-operated group and four between the sham-operated and kainic acid-treated animals. The comparison of the ADX, sham and kainic acid-treated groups with the ADX/kainic acid group, however, reveals 18 differentially expressed genes. These results indicate that the main mode of corticosteroid receptor-controlled gene expression in the hippocampus is interaction with other transcription factors (e.g. CREB, AP-1) and not primarily by binding to hormone responsive elements of corticosterone-specific genes. Furthermore, because corticosterone levels in the kainic acid-treated group were such that only the high-affinity MR was occupied, we suggest that the modulating effect of corticosterone on kainic acid-induced gene expression is mediated by the MR and not by the GR.

In conclusion, we will present data on the nature of some of the differentially expressed genes and discuss their possible role in hippocampal neurodegeneration.

793.2

SERUM RESCUES NEURONS FROM HYPEROSMOTIC-INDUCED PROGRAMMED CELL DEATH. **C. Dieckmann* and E.L. Feldman. Department of Neurology, University of Michigan, Ann Arbor 48109.**

SH-SY5Y neuroblastoma cells are a cloned cell line which ultrastructurally resembles developing neurons. These cells are a good model system in which to study the potential mechanisms which underlie neurotoxicity secondary to hyperglycemic, hyperosmotic exposure, as seen in diabetes. In the current study, we determined if serum could rescue neural cells from hyperosmolar induced growth arrest.

SH-SY5Y cells (9×10^4 cells/cm^2) were rinsed and plated directly in serum-free media $\pm$ 5, 20, 50, 100 and 300 mM mannitol. Cell number was measured at days 1, 2 and 3 by a colorimetric assay which detects reduction of the tetrazolium salt MTT. By day 2, 20 mM mannitol had significantly decreased SH-SY5Y cell number. The extent of growth arrest over time correlated with the severity of hyperosmolar exposure and was maximal at 300 mM mannitol. Serum rescued SH-SY5Y cells from glucose-induced growth arrest in a dose-dependent fashion with significant improvement in cell number within 24 hrs.

Programmed cell death (PCD), or apoptosis, is an active process which occurs when an essential signal is withdrawn or a noxious signal is introduced. In normosmolar media, withdrawing serum from SH-SY5Y cells did not precipitate PCD measured by flow cytometry. In contrast, approximately 25, 40, 60 and 80% of cells undergo PCD in serum-free media made hyperosmotic in 300 mM mannitol after 24, 48, 72 and 96 hrs respectively. Addition of serum rescued cells from PCD; indeed, only 6, 14, 16 and 20 % of cells underwent PCD at 24, 48, 72 and 96 hrs respectively after hyperosmotic exposure in the presence of 10% serum. Rescue was serum dose dependent and initially observed at 0.6% serum. Collectively, our results imply that a factor in serum can act as a neural osmoprotectant by rescuing cells from PCD. Sponsored by R29 NS32843 and an award from the Juvenile Diabetes Foundation International, Number 194130.

793.3

RELIABILITY AND VALIDITY OF THE PHYSICAL DISECTOR. G.J. Popken, P.B Farel*. Curriculum in Neurobiology and Dept. of Physiology University of North Carolina School of Medicine Chapel Hill, NC 27599

The analysis of many neurobiological phenomena requires accurate and reliable estimates of neuron number. The physical disector (Sterio, 1984) has been offered as an unbiased and efficient means to estimate neuron number. The disector method provides estimates of neuron number based on a small sample of cells, providing efficiency, but at a potential cost in reliability. In this study the reliability and validity of the disector method were investigated using frog dorsal root ganglia. Effects of variables related to tissue orientation, volume estimation and sample size were also considered. Recommended protocols for data acquisition by means of the physical disector were found to introduce variability in estimates of neuron number ranging from 0.7-95%. This variability could mask experimental effects or introduce spurious trends. Though recommended sampling protocols can lead to unreliable estimates, statistically reliable and valid estimates were achieved with this method when the sample size exceeded the recommended value by 3-6 times and when careful attention was paid to tissue preparation and volume measurements. Disector estimates were consistently lower than empirical estimates for ganglia cut parallel to the long axis of the dorsal root but not for ganglia cut transverse to this axis. Application of the physical disector thus requires empirical validation and careful consideration of variables that may be specific to the particular experimental situation. Supported by grants NS16030 and NS14899.

793.4

UPREGULATION OF BCL-2 FOLLOWING DEAFFERENTATION IN AVIAN NUCLEUS MAGNOCELLULARIS . L.A. Adams, D.M. Hockenbery and E.W Rubel.* Virginia Merrill Bloedel Hearing Research Cntr and Fred Hutchinson Cancer Research Cntr, Univ. of WA, Seattle WA 98195.

Interruption of VIIIth nerve activity in young chickens results in apoptotic cell death of 30% of the neurons in the ipsilateral nucleus magnocellularis (NM), the avian homolog of the mammalian anteroventral cochlear nucleus. The protooncogene bcl-2 blocks apoptotic cell death in many cell types, including neurons. To evaluate a possible role of bcl-2 in mediating cell death or survival in this system, we have used a novel antibody against chicken bcl-2 to examine the expression of the protein in NM cells at 3,6 and 12 h, and 1-5 d following unilateral cochlea removal (CR) in P11 chickens. In unoperated control animals, bcl-2 is expressed constitutively in NM cells and is localized predominately to the cell nucleus. By 3 h after CR, bcl-2 expression increases in a subset of ipsilateral(ipsi) NM neurons, and by 6 h there is an apparent increase in all ipsi NM neurons. Furthermore, at 6 h the subcellular localization of the protein has become predominately cytoplasmic. At 12 and 24 h, there are two distinct populations of ipsi cells, one with markedly lower bcl-2 levels and the other with increased protein levels in the cytoplasm; this pattern persists between 2 and 4 d. By 5 d, most of the surviving cells express low levels of bcl-2 and the subcellular localization has reverted to a predominately nuclear pattern. These results indicate that bcl-2 expression is upregulated as an early response to deafferentation injury, and suggest that those cells which survive are able to sustain elevated levels of bcl-2 during a critical post-injury period. During this critical period, bcl-2 may exert its protective effect by interacting with cytoplasmic constituents. (Supported by NIH grant DC00520).

793.5

ALTERED EXPRESSION OF BH1/BH2 FAMILY GENE(S) FOLLOWING RETINAL GANGLION CELL AXOTOMY. L. A. Levin*, K. Geszvain, R. W. Nickells. Department of Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, WI 53792.

Certain apoptosis-related genes, including *bcl-2*, *bcl-x*, and *bax*, share homology at the amino acid level in the BH1 and BH2 domains, through which they also interact. As part of a search for novel members of this family, we studied their expression in retinal ganglion cells after axotomy.

We designed degenerate oligonucleotide primers to the BH1 and BH2 domains, reducing multiplicity by eliminating nucleotides specific to human sequences. Total RNA was isolated from Long-Evans rat retinas at 1 and 4 days after intraorbital crush of the ipsilateral optic nerve; the contralateral retinas were used as a source of control RNA. Reverse-transcribed (RT) cDNA was amplified by the polymerase chain reaction and the products separated with a 1.5% agarose gel.

A single band of the predicted 160 bp length was seen in the control retinas; this monotonically decreased in retinas undergoing axotomy. There were no differences in levels of a control amplimer (S16 ribosomal protein). RT-negative controls demonstrated no banding. This suggests that expression of one or more members of this family decreases after axotomy, and may correlate with retinal ganglion cell apoptosis in this setting. We are currently testing this hypothesis with *in situ* hybridization studies. (Supported by NIH EY00340, Research to Prevent Blindness, and American Health Assistance Foundation.)

793.6

A NEW MINIMAL ESTIMATE OF THE MAGNITUDE OF CELL DEATH IN THE GANGLION CELL LAYER OF THE DEVELOPING RAT RETINA. L. Galli-Resta,* B. Margheritti and M. Ensini Istituto di Neurofisiologia CNR, Via san Zeno 51, 56127 Pisa, ITALY.

Overproduction of cells and the subsequent elimination of the excess are common features of nervous system development. In a typical brain region about twice as many neurons can be found in development than in adult life. However, as many investigators have already indicated, the number of dying cells could be higher than these figures suggest, since in many neuronal populations death begins when new cells are still appearing. A realistic estimate of the magnitude of cell death is missing for the lack of precise methods for assesment. However, by labelling cells according to their time of genesis with 5-bromo-2'-doexy-uridine (BrdU), we have been able to follow cells born in limited intervals of time and obtain a minimal estimate of the number of dying neurons in the rat retinal ganglion cell layer. Surprisingly, our data suggest that at least 5 times as many ganglion cells die than commonly believed, and a similar figure is obtained for displaced amacrine cells.

793.7

BASAL CELLS, IMMATURE NEURONS AND MATURE OLFACTORY RECEPTORS UNDERGO PROGRAMMED CELL DEATH IN THE RAT OLFACTORY EPITHELIUM. Thomas J. Mahalik*, Dept. of Cellular and Structural Biology. Univ. of Colorado Health Sciences Center. Denver, Colorado. 80262.

In the olfactory epithelium of the adult rodent olfactory receptor neurons are generated throughout the lifetime of an animal. Despite the continual generation of new neurons, there is no corresponding increase in the thickness of the epithelium. This means that cell death must occur in the epithelium to offset the continual generation of new cells. In the present study, a sensitive method to label nicked DNA in dying cells, was combined with immunocytochemistry to determine the identity of dying olfactory cells. In addition, the positions of apoptotic cells were mapped to provide additional information about the identity of dying cells.

Eight adult Sprague-Dawley rats were intracardially perfused with 4% paraformaldehyde. Sections through the epithelium were mounted on slides and were labeled with the TUNEL method. Sections were then labeled with antibodies against NCAM, keratin, GAP43 and OMP.

The double labeling experiments revealed that each of the olfactory cell types (i.e basal cells (keratin), immature neurons (GAP43) and mature receptor neurons (OMP)) undergo apoptotic cell death. The results of the mapping study suggest that apoptotic cell death occurs in the lower 1/3 of the olfactory epithelim. That is cell death is most likely to occur in basal cells and immature neurons.

793.8

***IN VIVO* EXAMINATION OF THE ELECTROPHYSIOLOGICAL AND ANATOMICAL PROPERTIES OF AN IDENTIFIED MOTONEURON COMMITTED TO DIE.** A. W. DeLorme°*, K. A. Klukas°, S. E. Fahrbach^, and K. A. Mesce°. Dept. of Entomology and Graduate Prog. in Neuroscience°, Univ. of Minn., St. Paul, MN 55108; Dept. of Entomology and Neuroscience Prog.^, Univ. of Ill. at Urbana-Champaign, Urbana, IL 61801.

In the abdominal ganglia of the moth *Manduca sexta*, pairs of motoneurons, the MN-12 cells, undergo programmed cell death within 4 days of adult emergence. Preliminary studies on the degeneration of MN-12's target muscle (DE-5) indicate that it degenerates early, 6 - 8 hours before the neuron is committed to die. In addition, it has been shown that there is a lag time of 8 - 14 hours from the time the MN-12 cells are committed to die, to when the first histological indications of somata deterioration are observed. Our aim was to determine if and when the anatomical and electrophysiological properties of MN-12 become altered in relation to the time of muscle degeneration and the 8 - 14 hour lag between commitment to death (at about 29 hours after adult emergence) and somata degeneration.

Neurobiotin-filled electrodes were used to examine the anatomical and electrical properties of the MN-12s. Filled cells were reacted with a Neurobiotin antibody conjugated to Cy-5, and viewed with a laser scanning confocal microscope. Thus far, we find no discernible differences in the electrical and morphological characteristics of MN-12s examined from 1 to 20 hours post ecdysis and those MN-12s older than 30 hours, even though the older MN-12s have lost their muscle targets and experienced the 'triggers' for their death hours earlier. Recordings and subsequent dye fills of both MN-12 cells in a single ganglion were sometimes obtained during cellular degeneration. We found that one of the MN-12s had a normal appearance while the other MN-12 lacked all arborizations. It appears that the MN-12 cells degenerate rapidly as the two cells are known to die in close synchrony at about 8 - 14 hours after the cell is committed to die.

793.9

PHOSPHORYLATIONS ANALYSIS IN CEREBELLAR GRANULE CELLS UNDERGOING APOPTOSIS. C. Galli, D. Mercanti, M.T. Ciotti, M. Hribal, L. Milazzo, C. Volonté* and P. Calissano.
Institute of Neurobiology CNR, Via K. Marx 43, 00137 Rome, Italy.

We have previously described and characterised cerebellar granule cells undergoing apoptosis following removal of high potassium (PNAS 90:10989-93,1993). The drop of intracellular calcium was recognised as one of the first events in the signal transduction of apoptotic message in this model (J. Neurosci. 15:1172-79,1995). In this study we have analysed by two dimensional gel electrophoresis the phosphorylation pattern of cerebellar granule cells undergoing apoptosis. The apoptotic neurons showed a pattern similar to control cells for the majority of protein product analysed. One single peptide bearing about 120kDa of molecular weight and an acidic isoelectric point showed an increase in 32P labelling triggered by shifting extracellular potassium from 25 to 5 mM. We followed the kinetic of the phosphorylative event from 0 to 2 hours after the triggering of apoptosis. The increase in 32P incorporation in the 120 KDa protein occurred in the first 20 min, a plateau was kept up to 1 hour and a slow decrease leading to basal control level was observed in the second hour of the apoptotic process. The serine threonine kinase inhibitor staurosporine selectively prevented the low potassium induced phosporylation of the 120kDa protein. The phosphatase blockers okadaic acid induced an overall augmentation of the proteins phosphorylation including the 120kDa peptide. Interestingly, forskolin and IGF-1, which block apoptosis in cerebellar granule cells, were able to prevent any change in the phosphorylation state of the 120kDa protein. These data confirm that an imbalance between kinases and phosphatases is an important step in the apoptotic biochemical pathway and describe a protein phosphorylation placed downstream the decrease of intracellular calcium in the pathway leading to neuronal programmed cell death.

793.10

DENDRITIC APOPTOSIS: A NEW MECHANISM FOR RESTRICTED NEURONAL DEATH. M. Sugimori* B.D. Cherksey and R. Llinás, Dept. Physiology & Neuroscience, New York University Medical Center, 550 First Avenue, New York, NY 10016.

While studying calcium concentration changes in Purkinje cell dendrites *in vitro* using calcium dependent fluorescent dyes, we noticed that certain manipulations resulted in the death of well defined dendritic segments. Upon further study, we found that iontophoresis of glutamic acid could trigger local release of calcium from intracellular stores even in the absence of extracellular calcium and in conditions where dendritic spikes were totally absent. When the iontophoresis injection was continuously repeated on to the same site (6 to 8 times), the calcium concentration increase became permanent and a lack of dendrite response to further iontophoresis was noticed. In order to determine whether the calcium concentration changes observed were triggered by the activation of a metabotropic glutamate ligand, most probably activating IP3 dependent calcium release from intracellular stores, we repeated these experiments after intracellular injection of heparin (500 mgms/ml). This protein prevented the secondary release of intracellular calcium during glutamatic iontophoresis.

We further observed that following glutamate iontophoresis to a given dendritic branch, the large release of intracellularly stored calcium did not, necessarily, spread to other branches within the period observed in a slice condition. These results are viewed as indicating localized release of calcium from intracellular stores may, under conditions, produce apoptosis of particular dendritic branches rather than the whole cell. There is evidence from neuropathology, especially in aging subjects, that such dendritic apoptosis is one of the main determinants of grey matter loss as opposed to the actual loss of whole neurons. Supported by NIA-AG09480.

793.11

MULTIPLE CED-3 AND CED-9 HOMOLOGUES ARE EXPRESSED IN THE MURINE NERVOUS SYSTEM. A.Sh. Parsadanian*, J.L. Elliott, and W.D. Snider, CSNSI, Dept. of Neurology, Washington University School of Medicine, St. Louis, Mo. 63110.

Recent investigations in vivo and in vitro have confirmed the central importance of ced-3 and ced-9 homologues in the regulation of neuronal survival. However, each of these families is complex and appears to contain many interacting members. Whether these molecules are expressed spatially and temporally in patterns appropriate to regulate neuron survival is unclear. We show here, using in situ hybridization, that the survival promoting ced-9 homolgues (bcl-2 and bcl-x), the death promoting ced-9 homologues (bax and bad) and the bcl-2 binding protein (BAG-1) are diffusely expressed in the murine peripheral and central nervous systems both during development and in maturity. Similarly, the ced-3 homolgue, nedd-2, is expressed by virtually all neurons at all developmental stages. In contrast, the ced-3 homolgue ICE is not expressed in the murine nervous system.

There are however, some developmental differences in expression patterns between the various family members. Temporally, mRNA for all of these molecules appears to decrease as animals mature, except for BAG-1. BAG-1 is expressed in low levels in embryonic mouse brain, then increases during early post-natal stages and into adulthood. In maturity, both ced-9 and ced-3 family members exhibit some cellular specificity in that expression is much more intense in neurons than in glial cells.

These results demonstrate that multiple ced-3 and ced-9 homologues are expressed in the murine nervous system, underscoring their central importance to regulating both naturally occurring neuronal death during development as well as neuronal survival in adult animals. These results also suggest that programmed cell death in all neurons may be regulated by similar intracellular mechanisms.

793.12

GENES ASSOCIATED WITH APOPTOSIS ARE EXPRESSED BY DYING MOTONEURONS AFTER NEONATAL AXOTOMY. R.W. Gerfen*[1], J.L. Elliott[1], E.M.[2] Johnson Jr., and W. D. Snider[1]. CSNSI, Dept. of Neurology[1] and Molecular Biology[2], Washington Univ. School of Medicine, St.Louis, Mo. 63110

Molecular mechanisms underlying neuronal programmed cell death are being clarified by the identification of genes which are associated with apoptosis in vitro. In order to assess the significance of these genes in an in vivo system, we asked whether facial motoneurons dying after neonatal axotomy exhibit morphological and molecular markers of apoptosis. After a right facial axotomy on post-natal day 1, newborn rat and mouse pups were sacrificed at 0.25, 1, 2, 3, 4, 5, 6, and 7 days. Hoechst staining revealed the presence of condensed and brightly fluorescent chromatin in facial motoneurons beginning 3-4 days after axotomy. Motoneurons exhibiting postive Hoechst staining were most numerous at days 4 -5 post axotomy, although they never comprised more than a few percent of facial neurons. Terminal transferase labeling using the Tunel method also demonstrated postive staining in facial motor neurons, again most abundant on days 4-5 after axotomy. Using ^{33}P labeled oligonucleotide in situ hybridization, we determined whether axotomized motoneurons in vivo exhibit changes in mRNA expression for transcription factors that have been linked to apoptosis in vitro. C-jun mRNA expression is heavily increased in facial motoneurons beginning at 24 hours after axotomy, remains elevated until day 3 and then declines. This increased c-jun expression appears uniformly in all axotomized motoneurons. In contrast, c-fos and NGF Ia mRNA expression begin to rise at 48-72 hours after axotomy, and remain intense even at 5 days post axotomy. Importantly, intense expression is limited to a small subset of facial motoneurons which appear shrunken and condensed. Levels of mRNA expression for other genes linked to apoptosis either decline in motoneurons post axotomy (JunD, cyclin D1, nedd-2) or are never expressed (Jun B, Fos B, ICE, Cyclin D2 & D3). These results confirm that motoneurons dying after neonatal axotomy, do so via apoptosis. In addition the temporal pattern of transcription factor expression is similar to what has been described in vitro (J. Cell Biol, 127:1717).

793.13

LONG-TERM SURVIVAL OF NEONATALLY LESIONED MOTONEURONS IN BCL-2 TRANSGENIC MICE. F. de Bilbao and M. Dubois-Dauphin* Dept. of Physiology, University Medical Center, 1211 Geneva, Switzerland.

We have previously reported that neonatal axotomized facial motoneurons which overexpress Bcl-2 were protected against neuronal death for a period of 7 days. Using the TUNEL technique, we have now observed that following facial nerve lesion in two days old wild type mice motoneurons die by apoptosis within 72 hours. In contrast, in transgenic pups apoptosis was not detected in lesioned facial motoneurons during this same period.

Furthermore we have examined the long-term survival of these lesioned facial motoneurons overexpressing Bcl-2. Twelve weeks after the lesion in two days old pups, 74% (n=3) of facial motoneurons survived axotomy in transgenic mice (when compared to the control nucleus), whereas in wild type mice all lesioned neurons had died during the first week after the lesion. The mean diameter of control motoneurons was 26 μm whereas lesioned surviving motoneurons were smaller (13 μm, $p< 0.001$). The nucleus size of control facial motoneurons was 13 μm and 9 μm in lesioned ones ($p< 0.05$).

These results suggest that Bcl-2 confers long-term survival to the lesioned facial motoneurons. Moreover, this survival may be independent of neurotrophic factors since we have not observed facial nerve regrowth at the level of the facial musculature. However, ipsilateral to the lesion, facial nerve axons were always presents in the portion of the facial nerve travelling in the brainstem of transgenic mice.

793.14

IMMUNOBLOT AND IMMUNOHISTOCHEMICAL ANALYSES OF ISCHEMIC NEURONAL INJURIES IN THE MONKEY HIPPOCAMPUS WITH REFERENCE TO CALPAIN ACTIVATION

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Calcium-activated neutral cysteine protease, calpain, is known to proteolyze fodrin or protein kinase C. However, the role of calpain in ischemic cell injuries has not been clarified. This study was performed to clarify the role of calpain in the development delayed neuronal death after transient brain ischemia in the hippocampal CA1 of the primates, using Westernblot and immunohistochemical analyses. Two male and two female monkeys weighting 7.5 to 8.5 kg were used. Monkeys were anesthetized with 1.0 to 2.0% halothane in a gas mixture of 40% oxygen and 60% nitrous oxide. Brachiocephalic artery and subclavian artery were exposed and occluded with vascular clips for twenty minutes. Rectal temperature was monitored throughout the experiment and maintained at 37°C with a heating sheet. Three antibodies that specifically recognize inactivated or activated forms of μ-calpain, and fodrin breakdown products were used. Increase of activated μ-calpain together with decrease of inactivated μ-calpain were observed especially in the perikarya of CA1 neurons after transient brain ischemia. In contrast, fodrin breakdown products, which were not observed in the control hippocampus, increased in all sectors of hippocampus after the ischemia. These results suggest that the activation of μ-calpain after brain ischemia plays an important role in the development of delayed neuronal death of the hippocampal CA1.

Key words ischemia, delayed neuronal death, calpain, hippocampus

793.15

Bcl-2 PREVENTS OXYGEN-INDUCED APOPTOSIS IN PC12 CELLS. T.Kubo*, Y.Enokido, T.Oka, N.Sato[1], Y.Uchiyama[1] and H.Hatanaka. Institute for Protein Research Osaka Univ., 3-2 Yamadaoka, and [1]1st Department of Anatomy, Osaka Univ. School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565, Japan.

The brain is one of the most energy consuming organs and exclusively depends on the aerobic energy metabolism using oxygen and glucose. Thus, the neurons are always exposed to oxidative stress in their long lives. It has been reported that the oxdative damage was deeply related to Parkinson's and Alzheimer's diseases. It is therefore important to investigate the mechanisms by which the oxdative damage brings about the neuronal death. We have reported that apoptotic neuronal death was observed when embryonic rat hippocampal neurons were cultured in a 50% oxygen atmosphere (*Neuroscience*, **57**:965-972 1993). To study the mechanisms of oxygen-induced neuronal apoptosis in molecular levels, we established the system using PC12 cells. When cultured in a 50% oxygen atmosphere, PC12 cells largely died within 3 days. This cell death was prevented by protein and RNA synthesis inhibitors and the chromatin condensation was observed in cells grown in a 50% oxygen atmosphere. These results suggest that this cell death is mediated by an intracellular active death program, so called apoptosis. The high concentrations of potassium (>26 mM) in culture medium also inhibited oxygen-induced apoptosis in PC12 cells. To characterize the oxygen-induced apoptosis further, we used PC12 cells overexpressing the proto-oncogene *bcl-2*, which has been reported to prevent apoptosis in various types of cells. A large number of the *bcl-2*-transfected cells survived in a 50% oxygen atmosphere for 3 days in contrast to those transfected with the control vector. These results strongly suggest that Bcl-2 prevents oxygen-induced apoptosis in PC12 cells.

This system should be useful for analyzing the molecular mechanisms of oxygen-induced neuronal apoptosis in detail.

793.16

THE TUMOR SUPPRESSOR PROTEIN p53 MEDIATES DNA STRAND BRAKES-INDUCED APOPTOTIC NEURONAL DEATH. Y. Enokido*, T. Araki, S. Aizawa# and H. Hatanaka. Institute for Protein Research, Osaka Univ., 3-2 Yamadaoka, Suita, Osaka 565, # Institute of Molecular Embryology and Genetics, Kumamoto Univ. School of Medicine, 2-2-1 Honjyo, Kumamoto, Kumamoto 860, Japan.

The tumor suppressor protein p53 serves as a critical regulator of a G1 cell cycle checkpoint and of apoptosis following exposure cells to ionizing radiation or DNA-damaging agents. Although the physiological function of p53 in the nervous system is still unknown, recent studies have shown that p53 is surely expressed both in the PNS and CNS. Especially, it was also reported that p53 induction is associated with neuronal damage in the CNS. In this study, we focused on the effect of DNA damage on the neuronal survival, and investigated the role of p53 in DNA damage-induced neuronal death by using p53 deficient ($p53^{-/-}$) mice. When mouse cerebellar granule neurons (postnatal day 15-16) were treated with cytotoxic drugs that cause DNA damage: etoposide, a topoisomerase II inhibitor; bleomycin, which cleaves DNA; cisplatin, which creates intrastrand cross-links; and mitomycin C, an alkylating agent, massive neuronal death was observed within 12-24 hr. In contrast, neurons from $p53^{-/-}$ mice showed evident resistance to etoposide and bleomycin but not to cisplatin and mitomycin C. Although $p53^{-/-}$ neurons were also resistant to the neurotoxicity of cytosine arabinoside (AraC), neuronal death induced by low K^+ medium was similarly induced in both wild type and $p53^{-/-}$ neurons. Furthermore, in cultures continuously labeled with 5-bromo-2'-deoxyuridine (BrdU) for 24 hr, neither wild type nor $p53^{-/-}$ neurons were labeled by BrdU. These results indicate that p53 is directly involved in DNA strand brakes-induced apoptotic neuronal death and it mediates the different intracellular death cascade from low K^+ medium-induced neuronal death.

793.17

Global ischemia induces several genes associated with apoptosis in the gerbil hippocampus. J. Honkaniemi*, S.M. Massa, M. Breckinridge and F.R. Sharp. Dept. of Neurology, University of California, Department of Veterans Affairs Medical Center, San Francisco, CA 94121.

We studied the effect of global ischemia on the expression of interleukin-1ß converting enzyme (ICE), *bcl-2*, *bcl-x*, and *bax* in the gerbil hippocampus. Using *in situ* hybridization *bcl-2*, *bcl-x*, and *bax* were all found to be expressed in CA1-CA3 pyramidal region and dentate gyrus of the normal adult gerbil. Following 5 or 10 min of global ischemia *bcl-2* and *bcl-x* mRNAs were induced 24h later in CA1 neurons whereas the expression of *bax* was unchanged. At 72h following global ischemia ICE mRNA was weakly induced in the CA1 region. At this time the expression of the other genes was markedly decreased in the CA1 pyramidal cells. Using DNA nick end labelling, neurons showing DNA fragmentation were mainly localized in the CA1 neurons, although 72 h after 10 min ischemia, labelled neurons were also observed in the cortex and the CA3 region. The *bcl-2*, *bcl-x* and *bax* oligonucleotide probes detected bands of expected size on Northern blots. However, the two ICE oligonucleotide probes used detected two additional bands of 3 kb and 1.5 kb in addition to the expected prominent 2 kb band. All three isoforms of *bcl-x* were expressed in normal hippocampus as demonstrated by RT-PCR. 24 and 72 h after ischemia, the relative mRNA levels of the large isoform (*bcl-*x_L) were decreased, whereas little change was seen in the expression of the short (*bcl-*x_S) and transmembrane deleted (*bcl-*$x_{\Delta TM}$) isoforms. The induction of *bcl-2* may tend to prevent apoptosis at 1 day. However, the decrease in the protective *bcl-*x_L compared to the apoptotic *bcl-*x_S may be an important factor leading to apoptotic death. This data suggests that after global ischemia CA1 neurons express a number of genes associated with apoptosis implying that the CA1 neurons undergo apoptosis-like death with activation of endonuclease and fragmented genomic DNA.

793.18

A MONOCLONAL ANTIBODY THAT INDUCES APOPTOSIS IN NEOCORTICAL NEURONS BINDS TO A NOVEL CLASS OF DEATH RECEPTOR L.T. Zhong, L. Noterpek, J. Oh, Y.L. Ruan, L. Butcher, K.F. Faull, A.L. Fluharty and D.E. Bredesen* UCLA Neuroscience Program, Los Angeles, CA 90024, La Jolla Cancer Research Foundation, La Jolla, CA 92037

Neuronal apoptosis during development and pathogenesis of degenerative diseases may be induced by both internal and external signals. In the process of searching for these death signals and their receptors, a monoclonal antibody, designated NAIM-1 (neural apoptosis-inducing monoclonal 1; 1 out of 13,000 clones screened), was found to induce apoptosis in some rat neural primary cultures and cell lines. The killing was not due to complement related cell lysis. Bcl-2, P35, and Cu-Zn SOD, gene products which were shown to block apoptosis induced by several but not all stimuli, prevented apoptosis induced by the antibody. Histochemical staining showed no antigen expression outside the central nervous system. Within the CNS, the antibody bound predominantly to cortical neurons, especially pyramidal cells of cortical layer V. The antigen that the monoclonal antibody recognizes is not a member of the FAS-TNF receptor family. Characterization of the antigen will be presented.

793.19

APOPTOSIS CONTRIBUTES TO ISCHEMIC BRAIN INJURY IN OCCLUSION/REPERFUSION MODEL IN THE CAT Z.S. Vexler*, I.V. Klimanskaya, T.P.L. Roberts, N. Derugin, J. Kucharczyk, A. Arieff. Neuroradiology Section, Departments of Oral Biology and Medicine, University of California, San Francisco, CA 94143, USA.

Apoptosis and necrosis, two morphologically and biochemically distinguishable forms of cell death, have been implicated in neuronal loss following cerebral ischemia. The purpose of this study was to evaluate the contribution of apoptosis in brain injury in relation to the degree of hypoperfusion, using the model of focal transient middle cerebral (MCA) occlusion in the cat.

The right MCA of three cats was occluded for 2 hours followed by 10 hours of reperfusion. Diffusion-sensitive and contrast-enhanced MRI were performed every two hours and maps of the apparent diffusion coefficient (ADC) and of cerebral microcirculation ($\Delta R2^*$) were constructed. At the conclusion of experiments, triphenyl-tetrazolium chloride (TTC) was perfused intracardially. 5 μm coronal sections anatomically matching those observed on MRI were cut from formalin-fixed brain tissue. Ischemic brain damage was established using TTC and H&E staining. Presence of apoptotic cells was evaluated in situ by direct fluorescence or immunoperoxidase detection of digoxigenin-labeled genomic DNA. The contralateral MCA territory served as a control.

By two hours of MCA occlusion, the ratio of peak ΔR_2^* effects in ischemic parietal cortex and basal ganglia was decreased (17±8%) compared to anatomically matched contralateral tissues, and brain edema (30-50% drop of the ADC) was detected. Upon reperfusion, a complete restoration of cerebral microcirculation was observed. A delayed decline of the cerebral microcirculation varied in different areas. Lack of TTC staining correlated well with areas of prolonged perfusion deficit on the contrast-enhanced MRI. Cells showing digoxigenin staining were present in basal ganglia ipsilateral to the occluded MCA, regardless of the degree of disturbance of the microcirculation during reperfusion. In the cortex, cells showing digoxigenin staining were preferentially exhibited in the perifocal areas of ischemic core which were moderately perfused during reperfusion. Digoxigenin labeling was found both in pycnotic nuclei, and in the nuclei with normal morphology as revealed by double-labeling with 4,6-diamidino-2-phenylindole (DAPI) or methyl green. Our data suggest different stages of apoptotic process in individual cells.

Apoptosis appeared to be an important early contributor to ischemia-induced cell death in cats with focal transient ischemia.

793.20

LOCALIZATION OF ICE-LIKE PROTEASES, ENZYMES ASSOCIATED WITH APOPTOSIS, IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM. R. Siman*, S. Trusko, R.J. DiRocco, V.R. Marcy and R.W. Scott. Cephalon, Inc., West Chester, PA 19380

Interleukin-1β converting enzyme (ICE) is a cysteine protease responsible for production of mature IL-1β. Recent molecular genetic and cell biological findings have implicated ICE and a family of homologous proteins as key mediators of programmed cell death via apoptosis. The role of apoptosis in neurodegenerative processes in the adult CNS and the involvement of members of the ICE family are not well-understood. We have used immunohistochemistry to map the distribution of members of the ICE family in the rodent CNS, and have investigated effects of neuronal injury on expression of ICE family members. Synthetic peptides were used in preparation of antisera predicted to recognize either all known members of the ICE family, or specifically react with ICE (p20 subunit) of human and mouse origin or with mouse Nedd2. In the gerbil brain, prominent ICE-like immunoreactivity was restricted to the neuropil of substantia innominata of the basal forebrain. Nedd2-like immunoreactivity, on the other hand, was broadly localized and abundant in most neurons throughout the neuraxis. Although present in the cytoplasm of neuronal perikarya, axons, and proximal dendrites, Nedd2-like immunoreactivity was most intense in neuronal nuclei. The immunostaining was specific; it was confirmed with a second antibody raised to a distinct domain, and could be preabsorbed with peptide immunogen. The effect of neuronal injury on protease content was assessed in gerbils subjected to transient global ischemia. Immunoreactivity for ICE and Nedd2 declined in hippocampal CA1 pyramidal neurons over 3 days, and began to appear in astroglia as they became reactive. These results indicate that ICE and especially Nedd2 could be involved in apoptotic mechanisms in adult central neurons, and suggest that one target of Nedd2 action may be the nucleus.

CEREBRAL CORTEX AND LIMBIC SYSTEM IV

794.1

CALBINDIN, CALRETININ AND NADPH-DIAPHORASE IN DIFFERENT CELL CLASSES IN LAYER I OF THE ADULT HUMAN NEOCORTEX. G.Meyer*,D.Galindo-Mireles,F.Carrillo-Padilla and R.Ferres Torres. Dept.Anatomia,Fac.Medicina,La Laguna, Spain.

Cajal-Retzius cells are considered the principal cell type of neocortical layer I. They are common during fetal life, but only a subpopulation survives into adulthood. We analyze here the distribution and morphology of neurons in layer I in 22 different cytoarchitectonic areas (primary sensory, motor and association cortices) of 10 human brains aged 27-96 years, by using calbindin (CB) and calretinin (CR) immunohistochemistry and NADPH-diaphorase histochemistry. Like the fetal forms, adult Cajal-Retzius cells have a long horizontal axon and dendrites restricted to layer I. They occur in all areas examined, but show a topographical preference for the bottoms of the sulci. Most Cajal-Retzius cells are CR-immunoreactive (ir), but a few ones express CB. A subgroup of Cajal-Retzius cells, located deep in the sulci around entering blood vessels, is moderately NADPH-d positive. A second class of large cells in layer I is CB-ir. These neurons, observed in the prefrontal and non-auditory temporal areas, have long smooth dendrites descending to layer III, and an apparently local axon in layer I. Their similarity with CB-ir neurons in deeper layers suggests that they do not belong to the group of Cajal-Retzius cells, but rather share the morphology, and possibly the developmental history, of neurons derived from the cortical plate.

794.2

POSTNATAL DEVELOPMENT OF LAYER IIIC PYRAMIDAL NEURONS IN THE HUMAN PREFRONTAL CORTEX: A RAPID GOLGI STUDY. H.B.M. Uylings (1), Z. Petanjek (1,2), I. Kostović (2).(SPON: European Neuroscience Association); (1) Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands, (2) Croatian Institute for Brain Research, Zagreb, Croatia.

We studied the dendritic development of layer IIIC pyramidal neurons in the region of Brodmann's area 9 of the human prefrontal cortex (PFC) in rapid Golgi stained sections using a 3-dimensional semiautomatic system developed at the Netherlands Institute for Brain Research. Quantitative analysis was performed on basal dendrites of 25 subjects ranging from newborn up to 91 years. Research questions are (a) which phases of developmental growth can be detected, (b) is dendritic overgrowth apparent in this crosssectional study, (c) which life-span alterations are detectable, (d) does the development of layer IIIC pyramidal dendritic processes take place later than those in layer V pyramidal neurons and (e) can data of this silver rapid Golgi study be compared with those of the mercury Golgi-Cox study. So far, the results show a rapid dendritic growth of layer IIIC pyramidal dendrites in the first year. The number of branches in basal dendrites did not increase further after the first month, but especially terminal segments elongate and as a consequence total dendritic length until 1-3 years. Somata size reached adult values around 1 year and, at the age of 6 years, two specimen showed overgrowth in size. In addition, a temporary exuberant number of spines on these pyramidal cells was detected during the period of 3-6 years (Petanjek et al., 1995). In this study no dendritic regression in normal aging was evident, although interindividual differences increased after the age of 50.

In this rapid Golgi study, the maturation of layer IIIC pyramidal cells ended no later (if not earlier) than that of layer V pyramidal cells (Petanjek et al., 1995). A similar conclusion was reached in our Golgi-Cox study (Koenderink et al., 1994). Finally this study indicates that after structural maturation of layer IIIC pyramidal dendrites, chemical maturation of these neurons will continue (Kostović et al., Neurosci. Lett. 90: 107, 1988).

Supported by the Ministry of Science, Croatia, and the Van den Houten Foundation, The Netherlands.

794.3

CALLOSAL NEURONS AND GLIAL CELLS IN THE DEVELOPING CEREBRAL CORTEX OF HUMAN FETUSES. L. deAzevedo, R. Lent and C. Hedin-Pereira*. Instituto de Ciências Biomédicas, UFRJ, 21941-590 Rio de Janeiro, and Instituto Fernandes Figueira, FIOCRUZ, 22250-020 Rio de Janeiro, Brazil.

We have studied the morphology of developing callosal neurons and glial cells in human fetuses, by implanting DiI crystals respectively into the corpus callosum and in the marginal layer of two premature fetuses deceased at estimated ages of 28- and 33-weeks gestation. Small, paraformaldehyde-fixed blocks containing the cingulate gyrus and the callosum were dissected and implanted with crystals of DiI. The blocks remained in fixative during 4-6 months before being cut coronally at 300 μm and counterstained with DAPI. Most callosal neurons were spiny pyramids located at different depths in the cortical plate, with well-developed basal dendrites, and apical dendrites that consistently arborized within the marginal layer. Other cell types were rare, such as bipolar and multipolar neurons, and inverted pyramids. Glial cells became labelled with DiI either by the pial crystals or by contiguity with labelled callosal fibers. Three morphological types could be discerned: (1) radial glia with processes attached to the pial surface but not to the ventricular wall; (2) glial cells with short apical processes, and (3) astrocytes. Results show that by 28 weeks gestation callosal neurons are advanced in their morphological differentiation and radial glial cells are transforming into astrocytes, suggesting that radial neuronal migration has ceased in the human cerebral cortex.

Financial support: CNPq, Finep.

794.4

CALLOSALLY-PROJECTING SUBPLATE CELLS IN FETAL HUMAN BRAINS. R. Lent*, L. deAzevedo and C. Hedin-Pereira. Instituto de Ciências Biomédicas, UFRJ, 21941-590 Rio de Janeiro, and Instituto Fernandes Figueira, FIOCRUZ, 22250-020 Rio de Janeiro, Brazil.

Subplate neurons have been implicated in the formation of cortical pathways of developing non-human mammals. To verify if these neurons project callosal axons in the developing human brain, and to study their morphology, we placed crystals of DiI into the callosal tract of two premature fetuses deceased after birth. Their estimated ages at death were respectively 28 and 33 weeks of gestation. Small, paraformaldehyde-fixed blocks of the cingulate gyrus containing the corpus callosum were dissected and implanted with crystals of DiI. The blocks remained in fixative during 4-6 months, before being cut coronally at 300 μm. Sections were counterstained with DAPI, and screened under a fluorescence microscope to identify the labelled cells and delimit the cortical subplate. Several neurons located within the subplate were detected in both brains, and were documented photographically or drawn through a camera lucida. Most of these neurons were smooth, bipolar cells with long, tangential dendrites spanning a large extent of the cingulate gyrus, and some had labelled axons that could be followed into the corpus callosum. A few were radially oriented bipolar neurons, multipolar cells with no particular orientation, and inverted pyramids. Results reveal that subplate cells in the developing human brain do project axons through the callosum. Furthermore, the characteristic tangential orientation of these cells' dendrites provides a favorable architecture for interactions with afferents.

Financial support: Finep, CNPq.

794.5

SEX DIFFERENCES IN RESTING CEREBRAL GLUCOSE METABOLISM IN MONKEYS. M. J. Raleigh*, W. P. Melega, S-C. Huang, S. Cherry, Michael T. McGuire, and M. E. Phelps. Depts. of Psychiatry and Molecular and Medical Pharmacology, UCLA School of Medicine, Los Angles, CA 90024-1759.

This positron emission tomography (PET) study examined the relationship between sex differences in local cerebral metabolic rates for glucose (LCMRGlc) and the emergence of gender differences in behavior in developing vervet monkeys. We documented LCMRGlc in nine regions of interest (ROI) in conscious monkeys. The seven male and eight female subjects ranged two to 12 months and lived in complex, species typical social groups. Sex differences in aggression, affiliation and play were not present prior to six months of age but became prominent between six and 12 months of age. Similarly there were no sex differences in LCMRGlc in any ROI before subjects were six months old. However between six and 12 months of age, females had higher LCMRGlc than males in the orbitofrontal, dorsolateral frontal, temporal, and parietal cortex by 21%, 23%, 25%, and 23% respectively. By contrast, there were no sex differences in LCMRGlc in the visual cortex, primary motor cortex, thalamus, cerebellum, or brainstem. Thus sex differences in LCMRGlc are more pronounced in cortical association areas than in other regions and emerge coincident with the appearance of sex differences in complex social behavior. Gender may be a critical variable in specifying the links between developmental changes in LCMRGlc and behavior. Supported by the Dana Foundation, the Veterans Administration, and the NINDS.

794.6

THE ROLE OF CELLULAR ACTIVITY IN THE DEVELOPING CEREBRAL CORTEX. G.Magowan, and D.J. Price*. Department of Physiology, University Medical School, Edinburgh EH8 9AG, U.K.

An *in vitro* organotypic co-culture system was used to examine the development of the thalamocortical system. In culture embryonic day 15 thalamus innervates postnatal day 6 cortical explants and axons terminate within layer 4, as *in vivo*. Our hypothesis is that the release of neurotransmitters contributes to the determination of cell fate and target selection within this co-culture system. Neurotransmitter release may require or induce fluctuations in activity.

We either added tetrodotoxin (TTX), a sodium channel blocker, or increased potassium levels (to depolarise cells) in the developing co-culture system. TTX (10^{-6}M) resulted in increased thalamic outgrowth and eliminated the recognition of layer 4 by thalamic axons. KCl ($5x10^{-3}$M) resulted in excessive thalamic and cortical outgrowth and abolished target recognition. TTX and KCl treated co-cultures were sectioned and Nissl stained to reveal effects on cell viability. Preliminary results indicate that TTX enhances cortical cell viability, perhaps by reducing glutamate induced cell death in these co-cultures.

These experiments support a role for activity in the determination of cell fate and target recognition in the cortex. We are currently investigating whether neurotransmitters are involved in these processes, by adding specific receptor antagonists to the co-culture system.

794.7

POSTNATAL DEVELOPMENT OF ANATOMICAL AND ELECTROPHYSIOLOGICAL PROPERTIES OF LAYER I NEURONS IN RAT NEOCORTEX. Fu-Ming Zhou* and John J. Hablitz Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

Layer I and Cajal-Retzius (CR) cells are thought to be important in neocortical development with the CR cells thought to mature very early. Using whole cell patch clamp techniques and intracellular biocytin staining, we have studied the postnatal development of layer I neurons in the rat neocortex. Physiologically, in the first postnatal week, all layer I neurons had low resting membrane potentials, high input resistances and long membrane time constants. Action potentials (APs) were short in amplitude and long in duration. The firing frequency attained in very young cells was lower than in older ones. These parameters matured rapidly during the first 10 postnatal (PN) days. Very young layer I neurons had a prominent hyperpolarization-activated depolarizing "sag" which decreased with age. Morphologically, within the first postnatal week, bipolar horizontal neurons were prominent in layer I. These cells had very simple axonal arbors which did not extend into deep layers. Starting around PN7, these horizontal cells declined in number. Around PN10, nonpyramidal neurons with diverse morphology became the main neuronal components in layer I. Axonal collaterals of these cells were extensive and formed elaborate arbors, reaching into deep layers. These results suggest that 1) membrane properties of rat neocortical layer I neurons, including CR cells, are very immature at birth, 2) CR neurons do not show significantly earlier maturation in membrane properties than other layer I neurons, and 3) both membrane and morphological properties of layer I neurons mature rapidly in first two postnatal weeks.

794.8

DUAL COMPONENT mEPSCs IN LAYER I NEURONS OF RAT NEOCORTEX. John J. Hablitz* and Fu-Ming Zhou, Neurobiology Research, University of Alabama at Birmingham, Birmingham, AL 35294.

Dual component miniature miniature excitatory postsynaptic currents (mEPSCs) involving NMDA and AMPA receptors have been described in neocortical pyramidal cells. Using whole-cell patch clamp techniques and direct visualization of neurons, we examined the properties of mEPSCs in enigmatic layer I neurons. mEPSCs were recorded from all the layer I neurons tested. mEPSCs were prominent, occurring at rates of up to 10 Hz. In normal extracellular solution containing 1.3 mM Mg^{2+}, the inward mEPSCs consisted of fast and slow components. The slow component was absent at -70 mV, became apparent at -30 mV, was outward at +60 mV, and did not shown significant rectification. In Mg^{2+}-free solution, the slow component was observed even at -70 mV. D(-)2-amino-5-phosphonovaleric acid (D-APV) at 20 μM completely blocked the slow component indicating mediation by NMDA receptors. At -70 mV, the fast component had a rise time of about 1 ms, decayed with a single exponential time course of about 2.5 ms and was blocked by CNQX, suggesting mediation by AMPA receptors. In all layer I neurons tested (N=20), the fast component never reversed polarity even at holding potentials as positive as +80 mV. Thus, synaptic AMPA receptors on layer I neurons were highly inwardly rectifying. Bath-applied kainate (30 μM) induced an inwardly rectifying current which was blocked by CNQX. The degree of rectification of kainate responses was less than for mEPSCs. Layer I neurons have dual component mEPSCs mediated by NMDA and AMPA receptors. Synaptic AMPA-mediated responses are highly inwardly rectifying perhaps reflecting lack of GluR2 subunits.

794.9

CHAOS CONTROL IN THE DEVELOPING HIPPOCAMPUS?. J.V. Sanchez-Andres*, L. Menendez de la Prida and S. Bolea. Dept. Fsisologia, Inst. Neurociencias, Univ. Alicante, Aptdo. 374, 03080-Alicante, Spain.

Intracellular recording in hippocampal slices from rabbit shows sequential changes in the spontaneous activity of CA1 cells along early post-natal development (days 0-20 after birth). The maturation can be described as four stages: 1. Aperiodical bursting is the predominant event just after birth, accompanied by aperiodical spiking. 2. Spiking becomes more frequent and periodic while the bursting frequency decreases. 3. Bursting disappears, while periodic spiking remains. 4. Spiking frequency decreases to the level usually recorded in adult animals. The transitions among patterns are not homogeneous in the CA1 field of every single subject, pointing to an asynchronic maturation of the neurons. Independently of this heterogeneity the majority of cells behave similarly (stage 4) by the end of the studied period.

The transition from stages 1 to 3 correlates with a reduction in the afterhyperpolarization following injection of depolarizing current. This transition has been modeled with a Hodgking and Huxley formalism, showing that the reduction in the afterhyperpolarization can account for the sequential change in the pattern.

The transition from the early aperiodic to the adult-like pattern can be theoretically analyzed in terms of interspike interval maps showing a progressive degree of organization.

We hypothesize that the maturation of the afterhyperpolarization constitutes the main element in the early maturation of the pattern, while the last stage would be a consequence of both the development at the network level and of the synaptic interactions. If this process constitutes formally a case of chaos stabilization or a dynamical bifurcation would require further studies.

794.10

ONTOGENY OF THE PAIRED-PULSE INDEX: A MEASURE OF THE DEVELOPMENT OF HIPPOCAMPAL DENTATE GRANULE CELL MODULATION. J.D. Bronzino,* J.H. Blaise, R.J. Austin-LaFrance and P.J. Morgane. Dept. of Engineering and Computer Science, Trinity College, Hartford, CT 06106 USA.

Measures of hippocampal dentate granule cell field potentials recorded in response to paired-pulse stimulation of the perforant pathway applied over a range of interpulse intervals (IPI) were used to evalauate developmental changes in modulation of granule cell excitability. Population spike amplitude differences between the first and second evoked response of a pulse pair were used to construct a paired-pulse index (PPI) for chronically implanted, freely moving male Sprague-Dawley rats recorded at 15, 30, and 90 days of age. Comparison of the resulting PPIs indicated a distinct developmental progression involving the early inhibitory, facilitatory, and late inhibitory phases of granule cell modulation identified in adults. This progression was characterized by markedly less early inhibition (IPI = 20 and 30 msec.), a lower level of facilitation (IPI = 50 -150 msec.), and a complete lack of late inhibition (IPI = 300 - 1000 msec.) in 15 day old rats when compared to 90 day old animals. Values of the PPI obtained from 30 day old rats fell intermediate between the 15 day and adult groups. These results suggest that the PPI can be effectively used as an indicator of the level of functional maturation of intrinsic (i.e., GABA-ergic basket cells) and extrinsic (e.g., medial septum, median raphe, and locus coeruleus) afferent systems modulating dentate granule cell excitability. **This research supported by NSF Grant # BCS-9208128.**

794.11

ALTERATIONS IN THE PROPERTIES OF HIPPOCAMPAL NEURAL NETWORKS FOLLOWING EXPERIMENTALLY-INDUCED DECREASES IN NETWORK SIZE IN INFANCY. Karen L. Smith, Chong L. Lee and John W. Swann*. The Cain Foundation Laboratories, Department of Pediatrics, and Division of Neuroscience, Baylor College of Medicine.

A number of neurological disorders are thought to be characterized by a reduction of hippocampal pyramidal cell number in early life. Experiments were undertaken to assess the effects of reduced pyramidal cell number on hippocampal network operations in adulthood. Infant rats (3-5 days of age) were injected intrahippocampally with NMDA (5 nmoles in 50nL) and allowed to mature (50-60 days of age). Injection were made in area CA3. Histological examination of the hippocampus demonstrated a marked reduction in the size of the $CA3_A$ and $CA3_B$ hippocampal subfields. Hippocampal neuronal number was reduced. However, a well formed CA3 cell body layer was present and there was no evidence of reactive gliosis. Recordings from hippocampal slice preparations were undertaken in medium containing picrotoxin (20μM). These experiments assessed the status of pyramidal cell recurrent excitatory networks. Prolonged synchronized population discharges (3-10 sec in duration) occurred in NMDA treated hippocampus but were not observed in slices from contrateral hippocampus or vehicle injected controls. Field potential analysis and recordings from minislices suggest that the discharges arose from $CA3_C$. Paired intracellular recordings suggest that when discharges occurred in $CA3_A$ or $CA3_B$ neurons they were driven by events originating in $CA3_C$. Thus in areas of the CA3 region most altered by NMDA injections recurrent excitatory connectivity maybe poorly developed. In adjacent regions, hyper-interactive networks may exist.
Supported by NIH Grants NS18309 and NS11535.

794.12

ELECTROPHYSIOLOGICAL ASPECTS OF POSTNATAL DEVELOPMENT OF RAT NUCLEUS ACCUMBENS *IN VITRO*. M. Belleau and R.A. Warren*. Centre de recherche Fernand-Seguin, Montréal, Québec, H1N 3V2.

The nucleus accumbens (nAC) receives important glutamatergic input from several limbic structures. Each of these structures innervates the nAC with a characteristic pattern. These patterns are presumably achieve in part through the mutual interactions between these fibres within the nAC during development. In addition, the interactions between these different inputs may have global effects on nAC development itself. As a first step to study the influence of glutamatergic input on the development of the nAC, we have characterized the physiological properties of nAC neurons and of the hippocampal innervation of the nAC during postnatal development in rat *in vitro*.

Whole cell recording of nAC neurons was performed in parasagittal 400μm thick slices from animals aged from less than a week up to 60 days. A unipolar tungsten microelectrode was used to electrically stimulate hippocampal fibres.

Young nAC neurons displayed high input resistance, long membrane time constant, slow action potential and a poor capacity to fire repetitively as compare to neurons from older animals. The stimulation of the fimbria usually produced a dual excitatory postsynaptic potential presumably consisting of a non-NMDA and of a NMDA component in young nAC neurons. During the second postnatal week, a third late inhibitory component appears which presumably depends upon the maturation of nAC intrinsic circuitry to be expressed.

These results show that glutamatergic input is present early during postnatal life and, as a consequence, that it may have an important influence on the normal development of nAC.

VISUAL CORTICAL DEVELOPMENT II

795.1

INTRINSIC INTERLAMINAR CONNECTIONS IN AREA V2 OF MACAQUE MONKEYS ARE ALTERED AFTER MONOCULAR DEPRIVATION. I.Stepniewska[1], J.H. Kaas[1], and M. H. Tigges[2]*. Dept. of Psychology[1], Vanderbilt University, Nashville, TN 37240 and Yerkes Reg. Primate Res. Ctr[2]. of Emory University, , Atlanta, GA 30322

We studied the organization of intrinsic laminar connections in V2 of macaque monkeys monocularly deprived from birth, either by surgical removal of the lens (aphakic group) or by wearing a black contact lens (occluded group). Connections of cortical layers in these 2 groups and in control non-deprived monkeys were determined by making 100-200μm injections of the Fluororuby under visual control into approximately 2 mm thick cortical slices incubated 3-4h in a chamber with oxygenated Ringer's solution and then cut sagittaly into 50μm sections. One series of sections was used for fluorescent microscopy, alternate sections were reacted for CO and Nissl substance to reconstruct the laminar borders. All injections produced narrow radial bands 200-300μm in diameter of dense label within columns extending across cortical layers. Other features of transported label depended on the laminar location of the injection. Injections in supragranular layers produced the densest label in layers 2/3 and 5 that was often laterally offset from the injection's axis. Label in layers 4 and 6 was always more confined. Injections in infragranular layers led to dense, widespread label in layers 5/6 and more confined label in layers 2/3. Monocularly deprived monkeys exhibited differences from normal in density and lateral extent of horizontal connections. In comparison with controls, horizontal connections were denser in occluded and sparser in aphakic monkeys. They were unusually widespread in occluded monkeys, extending laterally up to 4mm, and often unevenly distributed, forming zones of dense and sparse label. Conversely, the extent of horizontal connections in aphakic animals was restricted in comparison with control (approx. 0.5 and 1mm from the injection site, respectively). These data show that deprivation effects can be traced into association cortex, past the geniculostriate system. {Supported by EY-09737; RR-00165 and EY-02686}.

795.2

DIRECTION SELECTIVITY OF CORTICAL NEURONS IN CATS REARED WITH CONVERGENT STRABISMUS. S. Hatta, Y.M. Chino, & E.L. Smith III*, College of Optometry, University of Houston, Houston, TX 77204-6052

Early discordant binocular visual experience disrupts the signal transfer characteristics of LGN X-cells in cats (Chino et al., *PNAS*, 1994). Specifically, contrast sensitivity of LGN units is reduced relative to their retinal inputs and substantial delays in signal transfer occur in some, but not all, LGN units. According to the 'linear summation model' for direction selectivity in simple cortical neurons (Reid & Shapley, 1991; Albrecht & Wilson, 1991; Jagadeesh & Ferster, 1993), our results in the LGN predict that the direction selectivity of simple cells is likely to be abnormal in strabismic cats. To test this prediction, unilateral convergent strabismus was surgically induced in 3-week-old kittens. Upon their maturity, extracellular single-unit recording techniques and stimulation methods with drifting or contrast-reversing sine wave gratings (24% contrast, 1.6 Hz & 3.1 Hz) were used to measure the direction selectivity of striate simple cells. We found that direction selectivity determined with drifting gratings was significantly reduced in strabismic cats compared to that in normal control cats. The reduction was directly related to alterations in the spatio-temporal summation characteristics of afferent signals revealed by contrast reversing stimuli. The results suggest that functional anomalies in LGN relay cells may be closely associated with the development of anomalous receptive field properties in cortical neurons. Supported by NIH Research Grants EY-08128, EY-03611, & RR-07146.

795.3

GENICULOCORTICAL AFFERENT ARBORS IN BINOCULARLY DEPRIVED KITTENS. A.Antonini and M.P.Stryker*, Keck Center for Integrative Neuroscience, Dept. Physiol., Univ. California, San Francisco, CA 94143-0444

In the kitten as little as 6 days of monocular deprivation by lid suture (MD) during a critical period causes a remarkable remodeling of the geniculocortical projections serving the deprived eye (Antonini & Stryker, 1993). These plastic changes correlate with physiological changes in MD animals in which visual cortical neurons become almost completely unresponsive to the deprived eye. These physiological and morphological changes may be due either to activity-dependent competitive interactions between the projections serving the two eyes or to disuse. We addressed this question by analyzing the morphology of geniculocortical arbors in binocularly deprived kittens in which the afferents serving the two eyes were equally deprived and subject to disuse, and neither had a competitive advantage.

Kittens were deprived by binocular eye-lid suture (BD) for one or two weeks prior to perfusion at 6 weeks of age. The arbors were anterogradely filled with Phaseolus lectin iontophoresed into the main laminae of the lateral geniculate nucleus. The lectin was visualized immunohistochemically, and single geniculocortical arbors were serially reconstructed in 3-dimensions. BD geniculocortical arbors were altogether indistiguishable from arbors in normal kittens or non-deprived arbors after short-term MD. They were significantly different from deprived arbors in the total length (mean: 15728 *vs* 7744μm, $p<.001$), number of branch points (mean: 150 *vs* 57, $p<.001$) and maximal density (mean: 78 *vs* 49μm/1000μm^2,$p<.02$). These results support the notion that competitive mechanisms rather than disuse are responsible for gross morphological remodeling of geniculocortical arbors.
Antonini A. & Stryker. MP, (1993) Science 260, 1819; Supported by NIH grant EY02874

795.4

ROBUST TWO-DAY OCULAR DOMINANCE PLASTICITY REVEALED BY SINGLE-UNIT RECORDING AND INTRINSIC SIGNAL IMAGING OF KITTEN AREA 17. TK Hensch*, MC Crair, ES Ruthazer, M Fagiolini, DC Gillespie, and MP Stryker. Dept. of Physiology, Univ. of California, San Francisco, CA 94143.

Occluding vision through one eye during a critical period in early life results in a loss of responses to the deprived (D) eye in visual cortex. D-eye geniculocortical arbor rearrangement appears to account for some of this loss, since their size is reduced by about half following 6 days of monocular deprivation (MD) (Antonini & Stryker Science '93). Anatomical effects of 4 days MD are less pronounced (A & S, pers comm), however, and varying degrees of plasticity in striate cortex have been noted for much shorter periods of deprivation. Rapid MD effects may reflect an important transitional state when D-eye afferents are anatomically present but functionally ineffective. We, therefore, investigated whether a brief MD of 2 days can consistently produce a loss of functional connections from the D eye to area 17.

Physiological recordings were carried out blind to the status of monocular vision initiated between postnatal day 27 to 31. During 48 hours of eyelid suture, kittens were either returned to their home cage or handled throughout the day to ensure that they remained alert. Ocular dominance of single units was profoundly shifted in both cases (CBI=0.10; n=107 cells, N=2 animals, and n=177 cells, N=3 animals, respectively). Robust orientation maps of intrinsic optical images from the same regions of cortex were acquired for the non-D eye but never observed for the D eye (N=4/4 cats). Our earlier work has shown that two potential initiators of plasticity, metabotropic glutamate receptors and nitric oxide synthase, do not mediate effects of 5-day MD. Similarly, neither MCPG (50mM) nor NOArg (22mM) affected the shift in single-unit responses (CBI=0.12, n=50 cells each) or the disappearance of D eye orientation maps following two days of eyelid suture.

Thus, powerful two-day ocular dominance plasticity at the peak of the critical period is indistinguishable by these analyses from week-long MD effects.

795.5

VISUALLY EVOKED POTENTIAL (VEP) ASSESSMENTS OF VISUAL FUNCTION IN A MONKEY MODEL OF TREATMENTS FOR HUMAN UNILATERAL INFANTILE CATARACTS. A.D. Aiyer, R.J. Brown, L. Stevens, and R.G. Boothe*. Division of Neurobiology and Vision, Yerkes Research Center, Emory University, Atlanta, GA 30322.

The purpose of these studies is to analyze treatment strategies for children with unilateral infantile cataracts. Infant rhesus monkeys received a lensectomy within 10 days of birth to mimic the surgical treatment that is typically given to human infants. Then infants were placed into two groups. The first (AFP-NP) wore extended wear contact lenses that focussed the aphakic eye to a far point and the fellow eye to a near point. The rationale for this treatment was to force usage of the aphakic eye when viewing far targets and the fellow eye when viewing near targets. Targets at mid distances would be expected to provide some binocular stimulation. The second group (ANP-PO) wore an extended wear contact lens on the aphakic eye that focussed it to a near point, and an occluder contact lens on the fellow eye for up to 90% of the time. The rationale for this group was to use part time occlusion to force usage of the aphakic eye. Animals in both groups were reared with the lenses until 1.5 years of age. Behavioral assessments with preferential looking methods during the rearing period indicated good acuity development in all eyes. However, VEP assessments at 3 years showed that the acuities in the aphakic eyes of the AFP-NP group had deteriorated substantially while acuities in the ANP-PO group were relatively stable. All animals exhibited directional motion asymmetry which is a marker for impaired binocular function, although strabismus was not as prominent in the AFP-NP group. It is concluded that selective defocus treatment methods are somewhat better at promoting binocular function, but part time occlusion methods are better at maintaining spatial vision. Supported by EY05975 and RR00165.

795.6

THE POSTNATAL DEVELOPMENT OF DENDRITIC MORPHOLOGY IN THE RAT VISUAL CORTICOCOLLICULAR PROJECTION. G. Prusky*, Department of Psychology, The University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4.

Previously, we have reported that rat occipital corticocollicular axons undergo predictable and significant reorganization during early postnatal development; a pattern that is altered by removing visual input. In the present study, we examine the growth of dendrites in this projection, during normal postnatal development and under visually-altered conditions, in order to elucidate the mechanisms that control the maturation of dendritic morphology.

During the first month of postnatal development, corticocollicular neurons were retrogradely labelled *in vivo* with injections of a fluorescent retrograde tracer into the superficial layers of the superior colliculus. Two -three days after these injections, between P5 and P30 at 5 day intervals, animals were perfused, their brains were fixed and sectioned, and cells were identified and intracellularly-injected with a horseradish peroxidase conjugate. The peroxidase was converted into a permanent, dark reaction product, the cellular dendrites were reconstructed and the number and centrifugal order of the branches were analyzed.

Between P5 and P15 corticocollicular dendritic arbor gradually increased in size and complexity. However, after P15 there was no significant alteration in dendritic morphology. This pattern of growth was not altered by neonatal enucleation, or by early eye opening at P7. These data suggest that the growth of corticocollicular dendrites may be guided by an intrinsic mechanism that is independent of environmental influence.

Supported by an NSERC grant to G.P.

795.7

DEVELOPMENT OF CAMP-LINKED METABOTROPIC GLUTAMATE RECEPTOR (mGluR2/3) AND DARK REARING INFLUENCE ON mGluRs (1, 2/3 AND 5) IN THE CAT VISUAL CORTEX. S.N.M. Reid*, C. Romano, T. Hughes, D. Devlin and N.W. Daw. Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven CT 06520-8061; Department of Ophthalmology and Visual Science, Washington University School of Medicine, St. Louis MO 63110.

Metabotropic glutamate receptors (mGluRs) are involved in NMDA-dependent and NMDA-independent synaptic plasticity. These receptors may also participate in sensory-dependent developmental plasticity in the visual cortex. mGluRs are coupled to various signal transduction pathways. In this study, we examined the developmental profile of cAMP-linked mGluR2/3. We also examined effects of dark-rearing on mGluR2/3 and PI-linked mGluRs (1 and 5) in the cat visual cortex. Immunohistochemical results show that the laminar distribution of mGluR2/3 changes with age. The change is different from either mGluR1α or 5. Besides laminar distribution, the quantity of mGluR2/3 also changes during postnatal development. In contrast to mGluR1 and 5, mGluR2/3 increases during the early postnatal stage. Dark-rearing increases quantity of the mGluR5 at the peak of the critical period for monocular deprivation; however, it has little effect on the quantity of mGluR1. As in the case of mGluR5, dark-rearing affects the laminar distribution of mGluR2/3. These results suggest that these receptors are probably involved in different developmental processes of the visual cortex. mGluR2/3 and mGluR5 are involved in sensory-dependent events, but mGluR1 is not. Supported by RO1 EY 00053 and HFSPO.

795.8

ALTERATION OF THE LTP/D MODIFICATION FUNCTION IN VISUAL CORTEX OF DARK-REARED RATS. A. Kirkwood* and M.F. Bear Dept. of Neuroscience and HHMI, Brown University, Providence, RI 02912.

Experience-dependent modifications of binocular connections in the immature visual cortex may employ the mechanisms of long-term potentiation (LTP) and long-term depression (LTD). Induction of both LTP and LTD require the activation of NMDA receptors. The magnitude and sign of plasticity appears to depend on the pattern and amount of NMDA receptor activation during conditioning stimulation. In visual cortex, the duration of NMDA receptor-mediated EPSCs undergoes a developmental decline that is prevented by rearing animals in complete darkness. We have tested the prediction that these changes in NMDA receptor function are paralleled by alterations in synaptic plasticity.

Slices of visual cortex were prepared from 4-6 week-old Sprague-Dawley rats that had been reared either in complete darkness or in a standard lighted environment. Synaptic responses to layer IV stimulation were recorded extracellularly in layer III. LTP was attempted with two patterns of high frequency stimulation. One pattern, theta-burst stimulation (TBS: 200 pulses delivered in 100 Hz bursts), evoked LTP of comparable magnitude in slices from dark reared (122 ± 5% of baseline at 20 min after TBS, n = 7 rats) and control rats (128 ± 4%, n = 6). However, the same number of pulses delivered in 20 Hz bursts caused significant potentiation in slices from dark-reared rats (118 ± 7%, n = 7), but little synaptic change in slices from light-reared rats (104 ± 3%, n = 6). Finally, low frequency stimulation (LFS: 1 Hz, 15 min), induced robust LTD in slices from control (82 ± 4% of baseline 30 min after LFS, n = 6), but not from dark reared rats (95 ± 3%, n = 7). These data suggest that patterns of synaptic activation are less likely to induce LTD, and more likely to induce LTP, in visual cortex of dark-reared animals. These results are consistent with the idea that the "modification threshold" -- the critical level of NMDA receptor activation at which synaptic modifications change sign from LTD to LTP -- varies as a function of the stimulation history of the visual cortex.

795.9

PROBING THE "PLASTICITY GATE" IN VISUAL CORTEX USING PAIRED-PULSE STIMULATION. H. Frank, A. Kirkwood, M.A. Paradiso* and M.F. Bear Dept. of Neuroscience and HHMI, Brown University, Providence, RI 02912.

Previous work in our lab has shown that high frequency stimulation of a site in the middle of the cortex (corresponding to layer IV), but not of white matter (WM), results in LTP of layer III field potentials (FPs) in visual cortical slices from adult rats. It has been hypothesized that inhibitory circuitry within or deep to layer IV normally acts as a "plasticity gate": a filter that constrains the types of activity patterns that can gain access to the modifiable synapses in layer III. In this study we have used paired-pulse stimulation to better understand the different patterns of synaptic activation that result from high-frequency stimulation of WM and layer IV. Extracellular FPs were recorded in layer III as different sites were stimulated. In each experiment, paired-pulse stimulation was applied to the WM while systematically varying stimulus strength and inter-stimulus interval (ISI). Then, while keeping the recording electrode in a fixed position, the stimulating electrode was moved to layer IV and the experiment was repeated. Stimulation of either site generally resulted in paired-pulse suppression (PPS) of the test response relative to the condition response, particularly at short ISIs (20-80 ms). Across these ISIs, PPS increased as stimulation intensity was increased, but this effect was far greater for white matter stimulation than for layer IV stimulation. With a 40 ms ISI, for example, low intensity stimulation of WM resulted in PPS of 89.1 ± 9 (test as % of condition response) compared with 92.6 ± 4 after stimulation of layer IV. However, at stimulation intensities yielding a FP ≥ 50% of the maximum, there was much larger PPS from WM stimulation (54.8 ± 11) than from layer IV stimulation (83.8 ± 6). The difference between WM and layer IV PPS at high stimulation intensities is significant at $P < 0.02$ (n = 8). An interpretation consistent with these data is that WM stimulation at high intensities recruits inhibition that is bypassed by stimulating layer IV.

795.10

SEQUENCE ANALYSIS OF cDNA CLONES WHOSE EXPRESSION IS ENHANCED WHEN THE VISUAL CORTEX DISPLAYS ENHANCED ACTIVITY-DEPENDANT PLASTICITY S.S. Prasad*[1], J. Hankins[1], D. Mitchell[2] and M.S. Cynader[1]. [1]Dept. of Ophthalmology., Univ. of British Columbia, Vancouver, B.C., Canada V5Z3N9 and [2]Dept of Psychology, Dalhousie University, Halifax, N.S., Canada.

It is now well established that the functional capabilities of the adult cerebral cortex are not fully mature early in postnatal life. The experience-dependant modifications of cortical connectivity and physiology in kittens have a well defined critical period which peaks at about 30 days of age. This critical period is input-dependant and can be prolonged into adulthood by restricting visual input to the cortex. This critical period for ocular dominance plasticity can be greatly prolonged simply by rearing kittens in the dark throughout the naturally-occuring critical period.

Previously we reported our initial analysis of a subtracted cDNA library comparing the 30 day old kitten visual cortex relative to the adult visual cortex. This library was screened with subtracted probes from the visual cortices of dark reared older kittens (4 months of age) that were well past the peak of their chronologically-defined critical period as well as normally reared adult animals. Together these experiments identified twenty six cDNA clones that hybridized with high intensities to both the subtracted probes for the visual cortex of young kittens, and also those of dark-reared older animals. Northern blot hybridization confirmed overexpression in kitten vs cats for ten of the clones, the other were too rare to be detected. Two of these cDNA clones detected signals only in the 30 day old kitten visual cortex . We have determined the partial nucleotide sequences for all of the differentially expressed cDNA clones in an attempt to identify any known homologies. Although four of the ten sequenced cDNA clones show sequence homologies to submitted human expressed sequence tags, our results indicate that majority of these clones have not been examined with respect to their actual function in the brain development. In order to examine the structural domains of these cDNA clones, we are screening the cDNA libraries to obtain the larger cDNA clones. Our current focus will be on those cDNA clones which are uniquely expressed in the kitten and older dark-reared visual cortex relative to the adult visual cortex.

795.11

INVOLVEMENT OF PROTEASES IN OCULAR DOMINANCE PLASTICITY IN KITTEN VISUAL CORTEX C.B. Griesinger* and C.M. Müller, Max-Planck-Institute for Developmental Biology, Tübingen, Germany

Ocular dominance stripe segregation during visual cortical development relies on selective growth and retraction of the initially overlapping geniculocortical afferents from either eye. As suggested by recent *in vitro* studies, the molecular mechanisms underlying such axonal remodeling may include the action of proteases, such as the plasminogen-activator/plasmin system (*Pittman et al., J.Neurochem., 64:566, 1995*) and thrombin (*Liu et al., PNAS, 91:10300, 1994*). We therefore investigated the role of proteases in ocular dominance plasticity by chronically infusing serine-protease inhibitors into the visual cortex of kittens by means of osmotic minipumps. Coincidently we performed monocular deprivation (MD) or reverse occlusion (RO) for 7 days. Thereafter we electrophysiologically assessed ocular dominance (OD) distribution, orientation selectivity and response strength. Leupeptin (100mM; all concentrations refer to pump reservoir) did not influence the OD shift after MD. However, the reversal of OD after RO was significantly retarded by leupeptin infusion (10.9% reversal index compared to 76% in controls). Orientation selectivity and response strength were unimpaired. Since leupeptin exhibits strong inhibitory activity against plasmin, but only weak affinity for thrombin, we tested the highly potent thrombin inhibitors hirudin (50μM) and SDZ 217-766 (100μM; gift of Sandoz LTD, Switzerland) in additional MD experiments. While hirudin failed to influence OD plasticity, the low molecular weight inhibitor SDZ 217-766 attenuated the OD shift towards the experienced eye (47% binocularity versus 21.7% in controls). The results indicate a role for leupeptin-sensitive proteases in progressive plasticity after RO, most likely mediated by the plasminogen-activator/plasmin system, known to participate in axonal growth. In contrast, thrombin might contribute to regressive changes like synapse elimination during MD. Supported by the BMFT 0316902

795.12

FAILURE TO REROUTE OR COMPRESS THALAMOCORTICAL PROJECTIONS AFTER PRENATAL POSTERIOR CORTEX ABLATIONS J. K. Niederer*, G. Maimon and B. L. Finlay. Developmental Neuroscience Group. Cornell U. Ithaca N.Y. 14853 The topographic specificity of the developing thalamocortical afferents resembles the adult pattern of connectivity from their initial entry into the cortical plate. In order to explore the nature of the cues producing this precise pattern of early innervation, we removed a large region of posterior neocortex on embryonic day 14, when the thalamic axons are just beginning to enter the subplate and before any innervation of the cortical plate has occurred.

On E14, two days before normal delivery, ablations of posterior cortex were made in hamster pups *in utero*. Under anesthesia, unilateral ventral incisions were made in the dams and 2-4 pups were exteriorized. Using the saggital and transverse sinuses as guides to the caudal and medial borders of the developing neocortex, we undercut variable amounts of the posterior neocortex with a small scalpel. Neonates were raised normally to weaning (P30). The cortex and thalamus of each animal were reconstructed to determine the extent of the cortical ablation and its subsequent effect on the thalamus. The cortex and thalamus contralateral to the ablation served as controls.

In the four experimental animals, we examined the following cortical areas, Oc1 (31-86% ablation), Te1 (0-89% ablation) and S1 (14-47% ablation), and compared their residual volume to their respective primary afferent thalamic nuclei. We found that the surface area of cortex removed was highly correlated with the volume of its remaining thalamic nucleus (r=0.87). This suggests not only that the thalamic axons are unable to innervate inappropriate cortical areas but also that they are unable to compress their projections into the remaining appropriate cortex even at this early developmental stage. Supported by NIH RO1 NS19245

795.13

DEVELOPMENT OF CORTICAL BINOCULAR DISPARITY TUNING AND CORTICOGENICULATE FEEDBACK

A. Grunewald* and S. Grossberg. Department of Cognitive and Neural Systems, Boston University, Boston, MA 02215.

The rapid processing of binocular disparity information requires highly tuned disparity-selective neural responses, yet at birth infants only show a coarse level of stereopsis. A model shows how complex cells can develop fine disparity tuning starting from coarse tuning. Competition across cortical complex cells determines a local winner, which is allowed to learn the pattern of activities that feed into the complex cell. Antagonistic rebound responses at the retina ensure that whenever learning occurs for one type of edge contrast at the complex cell stage, learning also occurs for the complementary edge contrast. In this way, complex cells develop to be insensitive to the polarity of contrast. At the same time, complex cells learn to fuse only stimuli where both eyes have the same polarity of edge. Whenever a complex cell emerges as the winner, a top-down matching, or confirmation, signal is sent to the LGN. This confirmation signal stabilizes the learning process. When the confirmation signal matches the LGN activity patterns, then the matched LGN activities are amplified. A mismatch between the the confirmation signal pattern and the LGN pattern leads to a reduction of LGN activity, so that a new winner is picked at the complex cell stage. Feedback signals are disparity-tuned, because, whenever a winner is picked at the complex cell stage, learning also occurs in the feedback pathways. Thus the model explains the importance of corticogeniculate pathways for the self-organization of disparity tuning.

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795.14

A MODEL FOR THE DEVELOPMENT OF ORIENTATION SELECTIVITY BASED ON EARLY SPONTANEOUS ACTIVITY. B.A. Olshausen* and D.J. Field, Dept. of Psychology, Cornell University, Ithaca, NY 14853.

During early visual development, the retina exhibits spontaneous neural activity in the form of "waves" (Meister et al., *Science, 252*:939-943). It has previously been observed that these waves provide the type of spatiotemporal correlations that could help to refine topography within the LGN, as well as to segregate inputs from left and right eyes into different layers. Assuming that the patterns of activity in the retina are reflected to some degree in the LGN, the question then arises as to what role these waves play in cortical development, particularly since the waves contain oriented structure that could be used to develop oriented receptive fields in the cortex. In previous work we have shown that a learning algorithm that attempts to find a sparse coding of a set of images will develop wavelet-like receptive fields that are localized, oriented and multiscale when trained on natural scenes. Here, we show that when the learning rule is applied to scenes consisting of static snapshots of simulated low spatial-frequency waves, the receptive fields that emerge are oriented, localized, and low frequency. (By contrast, learning rules based on linear hebbian rules will converge to the principal components of the input stream, which are not localized and generally unoriented.) Once these receptive fields have been learned, the amount of training required on natural scenes in order to develop localized, oriented, multiscale receptive fields is substantially reduced (by at least a factor of 4). It is hypothesized that the retinal waves of activity provide the cortex with early "visual experience" that approximates the localized and oriented structure of the natural environment, and that by learning on these waves the cortex is put into a good initial state for later learning on natural visual input.

795.15

Correlation-Based Learning Model of Joint Orientation and Ocular Dominance Column Formation. E. Erwin* and K.D. Miller, W.M. Keck Center for Integrative Neuroscience, UCSF, San Francisco CA 94143.

Previously, we have shown that correlation-based competition between left- and right-eye inputs can lead to development of ocular dominance (OD) columns, while that between ON- and OFF-center inputs can lead to simple cell receptive fields (RFs) and orientation (OR) columns. Here, we study a similar model involving four input types, ON- and OFF-center inputs from left and right eyes. We assume time-invariant input correlations. We have determined the conditions for concurrent development of ocular dominance and orientation columns with preferred orientation varying continuously across OD boundaries. We are studying the relationships that then develop between the two columnar systems.

We assume symmetries between left and right and between ON and OFF. This leaves four independent correlation structures: those between input cells of same or opposite eye, of same or opposite center type. By a linear transform, the development equations decompose into four ***independent*** equations describing development of receptive fields and of maps of four synaptic weight combinations: (1) the sum of all four weight types (SUM); (2) left-eye minus right-eye strength (OD); (3) ON-center minus OFF-center strength (OR+); (4) ON-center minus OFF-center from left eye, plus OFF-center minus ON-center from right eye (OR−). Each weight combination develops independently under a distinct correlation function formed as a linear combination of the original four correlation functions. We call these functions C^{SUM}, C^{OD}, $C^{\mathrm{OR+}}$, $C^{\mathrm{OR-}}$.

Joint maps of ocularity and binocularly-correlated orientation preference develop in a large regime where (1) C^{OD} is positive over the arbor radius (radius of LGN cells converging onto a cortical cell), favoring monocular RFs; (2) the Fourier transform of $C^{\mathrm{OR+}}$ (or $C^{\mathrm{OR-}}$) has a single peak at a wavelength corresponding to 0.6–1 arbor diameter, favoring oriented RFs; (3) the principal eigenvalues associated with C^{OD} and $C^{\mathrm{OR+}}$ (or $C^{\mathrm{OR-}}$) are of comparable size and larger than the eigenvalues associated with the other two functions. These other two functions are otherwise largely unrestricted in structure.

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796.1

NON-INVASIVE DOPAMINE DETERMINATION BY REVERSED PHASE HPLC IN THE MEDIUM OF FETAL MESENCEPHALIC CULTURES. A TOOL TO ENRICH GRAFT CAPACITY PRIOR TO TRANSPLANTATION.

L. Studer*, C. Spenger, B. Bühler, L. Evtouchenko, and R.W. Seiler, Dept. of Neurosurgery, Univ. of Bern, CH-3010 Bern, Switzerland.

Free floating roller tube (FFRT-) cultures allow long term maintenance of human and rat fetal ventral mesencephalic tissue (Spenger et al., 1994; J. Neurosci. Methods 54:63-73) which permits in vitro characterisation of tissue prior to transplantation. Ventral mesencephali of E13 rat embryos (n=10) were divided into 4 equally sized pieces each and were individually prepared as FFRT-cultures. After 4, 8, 12 and 16 days in vitro (DIV) medium (1ml) of each culture was collected during routine medium change and immediately stabilised adding 50 µl of orthophosphoric acid (7.5%) with 0.22mg metabisulfite. Dopamine was extracted after ultracentrifugation by an analytical kit (Chromosytems, No. 5000) and probes were determined with reversed phase HPLC using electrochemical detection (ESA, Mod. 5011).

The mean dopamine concentration in medium derived from one culture increased from 21 ± 11 pg at day 4 to 58 ± 43 pg at day 12 and decreased to 39 ± 30 pg at day 16, (n=24 each). In all cultures devoid of dopamine after 4 and 8 DIV (12.5%) levels remained below detectability at 12 and 16 DIV. There was a substantial variation in dopamine levels of medium derived from cultures of different embryos ranging from 82 to 282 pg dopamine. Statistically higher dopamine levels were detected in medium derived from cultures of the rostral part of the mesencephalon compared to that from cultures of the distal zone. After 12 and 16 days in vitro between 78% and 81% of dopamine was localised in 50% of the cultures assessed. These results show the feasibility of non-invasive individual characterisation of FFRT-cultures of fetal rat mesencephalon prior to transplantation.

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796.2

HUMAN FETAL VENTRAL MESENCEPHALIC TISSUE CULTURES MAINTAINED *IN VITRO* FOR 7 TO 14 DAYS AND TRANSPLANTED TO 6-OHDA LESIONED RATS GIVE RISE TO GRAFTS RICH IN DOPAMINERGIC NEURONS.

Christian Spenger*, Lorenz Studer, Ljudmilla Evtouchenko, Beatrice Bühler, and Rolf W. Seiler, Department of Neurosurgery, University of Bern, Bern, Switzerland.

Free floating roller tube cultures of human fetal ventral mesencephalon of embryonic age (EA) 43 to 72 days post conception have been prepared (Spenger et al., 1994; J. Neurosci. Methods 54:63-73) and transplanted. After 7-14 days *in vitro* the mesencephalic tissue cultures were transplanted to the striatum of adult Wistar rats which had received unilateral injections of 6-hydroxy-dopamine into the nigrostriatal bundle 3-5 weeks prior to transplantation. Graft survival was assessed in tyrosine hydroxylase (TH) immunostained serial sections of the grafted brains between 7 to 27 days post transplantation

All transplanted animals showed large, viable grafts containing TH immunoreactive (ir) cells. The density of TH-ir neurons in these human fetal xenografts was 1949± 404 TH-ir cells / mm^3 (N=26). No significant difference was detected when TH-ir cell density of grafts derived from human foetuses EA 43 to 56 days post conception was compared to grafts from human foetuses EA 56 - 72 days post conception. This shows that ventral mesencephalic tissue from foetuses older than 8 weeks can successfully be cultured and transplanted when using the free floating roller tube technique. These results promote the feasibility of effective *in vitro* maintenance of fetal human nigral tissue prior to transplantation.

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796.3

NESTIN POSITIVE MULTIPOTENTIAL NON-PASSAGE EGF-RESPONSIVE NEUROSPHERES

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Epidermal growth factor (EGF) screens and supports multipotential neurospheres from either adult or fetal brains (Reynolds, et at '92), and fetal sympathetic ganglia (Silani, et al, '94). To simplify previous laborious procedures of dissociation and obtain a greater number of large size neurospheres, we adopt a new non-passage culture method of procuring EGF-responsive neuropsheres for a long period of time. Undifferentiated and differentiated features of the cells derived from these non-passage neurospheres were examined by immunocytochemistry.

Single cells from cerebral cortex, striatum and mesencephalon were obtained after enzymatic dissociation of gestational day 14 fetus of Sprague Dawley rats and cultured in flasks with DMEM/F12, N2 and EGF (20ng/ml). EGF was added every 4 days. No further passage of neurospheres was made. Then, 3 to 10 month-old neurospheres of cortex, striatum and mesencephalon were subplated respectively on chamber slides with Neurobasal medium and 2% fetal calf serum (FCS) for 14 days before cells were fixed and immunocytochemically stained with antibodies against nestin, proliferating cell nuclear antigen (PCNA), microtubule associated Protein 2 (MAP2), 5-HT, 5-HT1A receptor, glutamic acid decarboxylase (GAD), glial fibrillary acidic protein (GFAP) and S-100.

Average diameter of non-passage neurospheres was about 200 - 650 µm by 3 weeks in culture. By 3 months, some of neurospheres had reached a size of more than 1 mm in diameter. After being subplated, under the Neurobasal medium and 2% FCS, cells migrated out of the neurospheres and differentiated. Nestin positive cells were found mainly inside and near the neurospheres. The size of these cells was smaller than that of nestin-negative cells. Cells both inside and outside neuropsheres were proliferative as indicated by PCNA immunostaining. Immunoreactive 5-HT, GAD, 5-HT1A receptor, MAP2 were observed on the cells with neuronal morphology. Cells with glial morphology stained positive with antibodies against GFAP and S-100. Current results suggest that EGF-responsive non-passage neuropsheres possess nestin and continuously proliferate. They can give progenies of both neuron and glia even after 3 to 10 months in culture.

796.4

HUMAN NEURONAL NTERA-2 CELLS (NT2) IMPLANTED INTO MURINE SPINAL CORD MATURE AND INTEGRATE ALONG THE HOST ARCHITECTURE INDEPENDENT OF HOST AGE OR LOCATION

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NTera-2 cells, derived from a human teratocarcinoma, were differentiated into neuron-like cells (NT2N) with retinoic acid and implanted into the spinal cords of nude mice to investigate the influence of the host milieu on the grafted cells. NT2N cells were implanted in the dorsal, ventral or central cord in either midline or lateral locations. Post-implantation survival times were varied for neonatal, young adult and adult grafts. Morphologic characteristics of the grafted cells were assessed, including nuclear size and shape and outgrowth of processes into the host parenchyma. Maturation of neurites was determined by expression of adult forms of tau and highly phosphorylated high molecular weight neurofilament (NF-H). Phenotypic characterization was also assessed by a panel of antibodies to neuronal markers, neurotransmitters and neuropeptides. No differences in NT2N grafts were observed between hosts of different ages. The phenotype, nuclear size and shape of the cells remained consistent within post-implantation survival times regardless of graft location or age of host at implantation. The morphology of neurite outgrowth mirrored that of the surrounding host tissue in all grafts. Confocal analysis suggested graft/host synapses. Robust axonal outgrowth was observed in white matter tracts at all host ages where cells were implanted in or near host tracts. Interestingly, long term neonatal grafts did not show longer outgrowth when compared to adult grafts. This may reflect a changing responsiveness to environmental cues as the NT2N cells mature in vivo. These results suggest that the engraftment of NT2N cells follows the host architecture, but not necessarily the neurotransmitter phenotype of the host neurons. Thus, the cells may mature in a self-determined manner while using the existing framework of the host to guide outgrowth. These characteristics in a human neuronal cell line may be useful for the delivery of therapeutics targeted at neurological diseases as well as for investigation of basic science questions such as process outgrowth and pathfinding.

796.5

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE IMMORTALIZED MESENCEPHALIC CELL LINE, $1RB_3AN_{27}$

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The immortalized mesencephalic cell line, $1RB_3AN_{27}$ has several neuronal and dopaminergic properties that make it a candidate for neurotransplantation in Parkinson's Disease. In order to further characterize this cell line, we stained $1RB_3AN_{27}$ with antibodies directed against the neuronal marker, neuron specific enolase (NSE), the astrocytic marker, glial fibrillary acidic protein (GFAP) and a marker of neural progenitor cells, nestin. For positive controls, cultures of dissociated E15 mesencephalon containing both neurons and glia were used. Nestin immunoreactivity showed a filamentous pattern in $1RB_3AN_{27}$ and NSE showed more dense and diffuse staining. This pattern of staining was mirrored in the controls. In contrast, $1RB_3AN_{27}$ showed faint and diffuse immunoreactivity for GFAP which was unlike the controls. HPLC analysis of cell homogenates of $1RB_3AN_{27}$ revealed that in the presence of 40 µg/ml pargyline and 5 µg/ml 2-amino-6-7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropteridine, an analog of tetrahydrobiopterin, these cells contained 0.73 ± 0.04 ng / (10^6 cells) of dopamine. These findings are consistent with a neuronal and dopaminergic phenotype for $1RB_3AN_{27}$.

796.6

GENETIC METHODS FOR INCREASING THE VIABILITY OF FETAL MESENCEPHALIC NEURONS *IN VITRO* AND *IN VIVO*

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In an attempt to increase the number of viable dopaminergic cells following intra-striatal implantation of fetal ventral mesencephalic (VM) tissue, two different genetic techniques are being explored. First, we have constructed both recombinant adeno-associated virus (AAV) vectors and herpes simplex virus (HSV) amplicons containing the human *bcl-2* gene. These constructs were packaged with their appropriate helper viruses and the replication incompetent particles are currently being used to directly infect dissociated E12-14 rat VM cells prior to transplantation into unilaterally 6-OHDA lesioned rats. Graft viability and functional integration are evaluated by monitoring the reduction in amphetamine-induced rotation and by characterizing immunohistologically the cellular composition and gene expression within the graft. Second, in order to stimulate stable vector-mediated gene integration/expression in post-mitotic neurons the AAV inverted terminal repeats (ITRs) have been placed flanking a transgene within an HSV amplicon. ITR flanked transgene expression and integration is being evaluated in non-mitotic cells *in vitro* in the presence and absence of the AAV *rep* gene. Other methods of direct gene transfer into fetal neurons are being investigated, including transplacental delivery of plasmids.

796.7

VESICULAR RELEASE OF GABA FROM ENGINEERED AtT-20 CELLS. R.C. Weatherwax*, A. Sandrasagra, and A.J. Tobin. Departments of Physiological Science and Neurology, Brain Research Institute and Molecular Biology Institute, University of California, Los Angeles, CA 90095.

Benzodiazepines, barbiturates, volatile anesthetics, and alcohol all act on $GABA_A$ receptors. Because such GABA mimetics are widely used to treat anxiety, seizures, and movement disorders, direct delivery of GABA itself may be experimentally and therapeutically useful. GABA, a zwitterion, cannot cross the blood-brain barrier, but transplanted engineered cells offer a method for the *in situ* delivery of GABA in experimental models of movement and seizure disorders. With this possibility in mind, we have used retroviral vectors containing GAD_{65} and GAD_{67} cDNAs to create GABA-producing cell lines. We have studied several such lines, including a number from AtT-20 cells, a tumor of mouse anterior pituitary. These engineered cells produce appropriate GADs, GAD mRNAs, and GABA. Unstimulated cells release accumulated GABA at a basal level, as determined by HPLC analysis. Treatment with 8-Br-cAMP stimulates the GABA release, but depolarization with 50 mM KCl does not. Our data suggest that, in engineered AtT-20 cells, GABA accumulates in large, dense-core vesicles that contain ACTH. (Supported by NINDS, NS22256)

796.8

TRANSPLANTATION OF TWO HUMAN TYROSINE HYDROXYLASE PRODUCING NEURAL CELL LINES INTO THE ADULT RAT BRAIN. M. Heredia*[1], A.S. Riolobos[1], J. Yajeya[1], J.M. Criado[1], A. de la Fuente[1], M. Santacana[2] [1] Dept de Fisiología y Farmacología, Univ. de Salamanca, Spain, [2] Instituto Cajal (CSIC), Madrid, Spain,

BE(2)C-M17, a human neuroblastoma cell line, and NT-2, a human teratocarcinoma cell line with the tyrosine hydroxylase (TH) gene transferred (kindly provided by Dr. R. Robbins), were stereotaxically grafted in four different regions of the rat brain; i.e., cerebral cortex, cerebellum, striatum and amygdala. Rats (n = 55) received injections of suspended M17 or NT-2 cells in HBSS (8-16 x 10^4 cells). Controls received an injection of vehicle only. At 1, 4 and 8 weeks after implantation, rats were anesthetized and perfused with 10% formaldehyde. The brains were sectioned and immunostained for TH and GFAP. The grafted M17 and NT-2 cells survived up to two months post-transplantation. NT-2 cell grafts never appeared to revert to a neoplastic state. By contrast, the M17 grafted cells generated tumors within 15 days after transplantation in 30 % of the grafted rats, generally affecting the striatum and amygdala grafts. The cells stained positively for TH *in vitro* but failed to stain *in vivo*. At 1 week post-grafting the M17 and NT-2 cells were round. At 4 and 8 weeks post-grafting most M17 cells were polygonal and extended neurite-like processes, while NT-2 cells were round or fusiform without processes. M17 cell grafts were strongly vascularized by invading host brain capillaries.

Our results show that both cell lines can survive for at least 2 months after intracerebral implantation, but they differ with respect to their pathogenicity. While the M17 cell line showed tumorigenic potential, the NT-2 cell line did not. (Supported by MEC- PB 91-0066 and DGICYT).

796.9

Non Transformed Human Stem Cell Lines Survive And Integrate Upon Transplantation Into The Embryonic Rat Brain. $E.Cattaneo, #L.Magrassi, ^R.Galli, ^P.Frolichsthal, #S.Pezzotta, #G.Butti, @S.Govoni and ^A.L. Vescovi*, $Inst. Pharmacol. Sciences, Univ. of Milan; #Dept. of Surgery, Univ.of Pavia, Policlinico San Matteo; ^Neurol. Inst. "C.Besta", Milan; @Inst. of Pharmacology, Univ. of Pavia, Italy.

We previously reported that conditionally immortalized neuronal progenitors and primary neuroepithelial cells isolated from the E14 rat striatum survive upon transplantation into the embryonic rat brain and organize into clusters of cells that progressively lose antigenic markers characteristic of immature cells (Cattaneo et al.,Dev. Brain Res.,1994). In this study we adopted the same experimental procedure to study the in vivo behavior of EGF-responsive human stem cell lines isolated from the diencephalon of 10 week old fetuses. Human donor cells were subcultured for up to two years and were exposed to 1μM BrdU, 6 days prior transplantation. On the day of the transplant, cells were also loaded with DiI. The animals were sacrificed on postnatal day 1. Following vibratome and cryostat sectioning cells were found widely dispersed and integrated into the host brain. and processes were often visible as judged by the DiI signal. The chromatin inside the nucleus of the DiI labeled cells was also distinguishable after Hoescht staining due to its more dispersed nature and lack of prominent eterochromatic spots. Despite the heterologous nature of the donor cells, no cluster segregation of the transplanted cells was ever observed following transplantation. Clear cases of BrdU and DiI double labeled cells were visible. These results indicate that fetal transplant may represent a suitable way to test for the stability and differentiation potential of non-transformed human CNS stem cells (partially supported by the Alzheimer's Association PRG-94-057 to E.C. and by funds of the Italian Ministry of Health to A.L.V.).

796.10

HOST REGULATION OF PROTEIN EXPRESSION IN INTRASTRIATAL GRAFTS OF CONDITIONALLY IMMORTALIZED NEURAL CELL LINES.
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Conditionally immortalized neural progenitor (CINP) cells has proven to be interesting candidates in *ex vivo* gene therapy experiments. The cells survive and integrate extensively when transplanted to the adult rat brain. Although the grafted CINP cells differentiate predominantly into glial-like cells, only 10-15% could be shown to express glial markers by immunohistochemistry when grafted to the intact brain. To investigate if a possible pool of resting, non-reactive astrocytes was present among the transplanted CINP cells we designed a study where astrogliosis was induced two weeks post-grafting of the CINP cells. The cells used in this study was HiB5 and ST14A (of embryonic hippocampal and striatal origin, respectively) created by immortalization with a replication defective recombinant retrovirus carrying a temperature sensitive allele of the SV40 Large T-antigen. The cell lines were grown under standard cell culture conditions at 33°C. After labelling with 3H-thymidine (1μCi/ml) for 72 hrs, 100 000 cells/side were injected bilaterally into the striatum of intact, adult rats as a single cell suspension. Two weeks later the animals were unilaterally lesioned either with ibotenic acid (ST14A n=4; HiB5 n=5) at the transplant site or with 6-hydroxy dopamine (ST14A n=4; HiB5 n=6) into the ipsilateral medial forebrain bundle to induce terminal degeneration around the transplant. One week later the brains were processed for immunihistochemistry for glial acidic fibrillary protein (GFAP), vimentin (VIM) or OX-42, a marker of microglia and macrophages, and processed for 3H-thymidine autoradiography. The excitotoxoic lesion induced a strong astrogliosis with up-regulation of all the markers tested. The percentage of transplanted cells expressing glial markers increased from 9% to 32% for GFAP, from 3% to 16% for VIM and from 2% to 12% for OX-42. In the 6-OHDA lesioned animals the percentage grafted cells expressing GFAP increased from 9% to 35%.while no significant changes in VIM or OX-42 was observed. in addition the GFAP-positive grafted cells changed their morphological appearance towards that of reactive astrocytes. The findings suggests that transplanted CINP cells differentiate to a large extent, respond to host derived stimuli and may take active part in the brain's reaction to injury when transplanted into the adult rat brain. .

796.11

TRANSPLANTATION OF EGF-GENERATED NEURAL STEM CELLS IN A RAT MODEL OF FOCAL ISCHEMIA. L. Santschi, D.M. Rosenbaum, R.E. Gross, S. Rybak, J.A. Kessler*. Albert Einstein College of Medicine, Bronx, NY 10461.

Neural transplantation following brain injury is a potentially promising technique for promoting functional recovery. The present study examined the feasibility of transplanting a population of EGF-responsive neural stem cells into infarcted tissue and analyzed the graft efficacy in supporting behavioral recovery. Progenitor cells isolated from E16 - E17 mouse subventricular zone were mechanically dissociated and plated in serum free medium containing EGF (Reynolds and Weiss, Science 255:1707, 1992). After 5-6 DIV, spheres of mitotically active cells growing in suspension were harvested, labeled with the DiI derivative, CM-DiI (Molecular Probes Inc.), and utilized for transplantation into the caudate/putamen of ischemic rats. Focal ischemia was induced in S-D rats by a suture model to produce MCA occlusion for 30 minutes. The cells were grafted 2 weeks after ischemia. Viable cells were clearly detected 30 days following transplantation, the latest time examined. Although most cells remained near the site of infusion, there was some migration of cells into surrounding parenchyma. We are currently examining whether cell grafting ameliorates behavioral deficits due to the stroke. Nevertheless, the survival of progenitor cell grafts in ischemic brain suggests that cell grafting may provide a means of intervening therapeutically after stroke.

796.12

NEURAL PROGENITOR TRANSPLANTATION FOR HYPOXIC ISCHEMIC (HI) BRAIN INJURY IN IMMATURE MICE. K. I. Park*, F. E. Jensen, E. Y. Snyder Depts. of Neurology & Pediatrics, Harvard Med. Sch., Children's Hospital, Boston MA 02115.

We have demonstrated that immortalized, central nervous system (CNS) progenitors (or "stem-like cells") may replace gene products & neural cells in various models of neurodegeneration. The present study was designed to explore the therapeutic potential of CNS progenitors transplanted into the brains of immature mice subjected to focal HI (FHI) or global ischemic injury. We hypothesized that stem-like cells could prove efficacious in either of 2 ways: 1) They might engraft within brain regions subjected to HI injury & differentiate into injured neural cells; 2) They might provide factors, trophins, cell-to-cell contact signals, or support structures which could promote the survival or recovery of some of the host's own injured neural cells & circuitry. Unilateral common carotid artery ligation combined with hypoxia on postnatal day 7 (P7) reliably produced FHI injury in the CD1 mouse brain with extensive ipsilateral infarction while leaving the contralateral hemisphere intact. The animal received a transplant of C17-2 neural progenitor cells within the cerebral ventricle ipsilateral to the region of infarction on P11. At maturity, there was evidence of robust engraftment & reporter gene expression by donor derived cells within the ischemic region. The donor progenitors appeared to have migrated to the region of infarction & integrated into fairly extensive areas of the infarct. Some progenitors may have differentiated into oligodendrocytes & neurons, the 2 neural cell types typically lost or compromised by HI. Other cell types were astroglial, though there was no evidence of scarring. Therefore, one strategy for the therapeutic use of neural progenitors in HI injury might be cell replacement of degenerated neural cell types & another one may involve using such cells to express therapeutic, regenerative factors (either intrinsically produced by the cells or engineered to do so *ex vivo*) within areas of infarction.

796.13

TRANSPLANTATION OF CNS STEM-LIKE CELLS AS POSSIBLE THERAPY IN A MOUSE MODEL OF SPINAL CORD DYSFUNCTION.
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Spinal cord (SC) injury is characterized by a loss of neurons, glia, and myelin, and a disruption of neuronal pathways within the cord. Utilizing neonatal sciatic nerve transection as a model for depleting populations of segmental SC motor neurons (MN) in the mouse, we have sought to replace specifically those degenerated cells by transplantation of a v-myc immortalized, multipotent clonal CNS stem-like cell line. This cell line, C17-2, has been previously shown to differentiate into and replace in mature animals, cortical neurons that have been induced to degenerate (Exp. Neurol. 129:9, 1994). Preliminary studies demonstrate that C17-2 is capable of long term engraftment in the SC of sciatic nerve axotomized hosts, transplanted at 4 weeks of age, i.e. 4 weeks following injury which effectively eliminates 70% of the MNs in the ventral horn. Normally progenitors give rise to SC MNs only during fetal neural development. However in this experimental paradigm a subpopulation of these engrafted progenitors have differentiated into cells with a morphology, size, and anatomical location in the ventral horn characteristic of the degenerated MNs. These results suggest the feasibility of this lesion/transplantation paradigm to study the replacement of specific populations of injured neurons in the dysfunctional SC. Furthermore, these neural stem-like cells are capable of expression of exogenous gene products *in vivo*, allowing for the expression of candidate genes that might not only enhance differentiation and engraftment of these cells but also potentiate host SC function following insult through the regeneration of endogenous cells and circuitry. (Supported in part by grants from the APA and PVA).

796.14

EMBRYONIC STEM CELLS TRANSPLANTED TO THE ADULT BRAIN: TYROSINE HYDROXYLASE (TH) POSITIVE NEURONS DEVELOPED SPONTANEOUSLY AND BY TRANSFECTION WITH HUMAN TH GENE.
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Embryonic stem (ES) cells derived from mouse blastocyst and maintained in culture with leukemia inhibitory factor (LIF) were transplanted into the lateral ventricle, striatum, cerebral cortex and midbrain of adult rats and mice. Before transplantation they were subjected to 3 different treatments: a) retinoic acid (RA) treatment, b) transfection with a plasmid containing the human tyrosine hydroxylase (TH) gene, or c) left untreated. Rat hosts were immune suppressed with Cyclosporin A; mouse hosts were not immune suppressed. Untreated and RA treated cells produced grafts with variable numbers of differentiated TH-positive neurons and fibers. These were negative for dopamine-ß-hydroxylase (DßH). Effect of graft location on differentiation could not be unambiguously determined, but differences in TH cell numbers were associated with different treatments. RA treated cells produced grafts that were acetylcholinesterase (AChE) positive, while no untreated grafts expressed AChE. Transfected cell grafts showed high numbers of lightly TH-immunoreactive cells, however many cells also stained intensely TH-positive with extensive dendritic arbors and axonal outgrowth. Transfected cells and axons were DßH-negative. TH-positive axons from all ES cell grafts often exhibited varicosities, and graft axons from TH-positive cells in the striatum innervated the host striatum. These characteristics hold promise for use of ES cells as neural precursors and gene delivery vehicles for transplantation.

796.15

MOUSE EMBRYONIC STEM CELLS: IN VITRO MANIPULATION AND USE FOR INTRACEREBRAL TRANSPLANTATION.
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We have developed methods to induce differentiation of mouse embryonic stem (ES) cells at high efficiency into either neurons or skeletal muscle. We wanted to use mouse ES cells as a genetic screen for novel factors capable of inducing differentiation and as a gene delivery system for the central nervous system. Mouse ES cells were transfected with the human tyrosine hydroxylase (TH) gene behind the mouse phosphoglycerate kinase promoter. To alleviate problems with down regulation of expression, plasmid vectors were used. We have obtained several ES cell clones that exhibit long term expression of the TH gene in vitro (> 2 mos.) and maintain this expression in vivo after transplantation to the CNS. To use ES cells as a screen for differentiation inducing factors, we introduced cDNA expression libraries into ES cells, selected cells induced to differentiate and isolated plasmids present within the differentiated cells. In this way, we screened large numbers of genes for involvement in cellular differentiation. One full length cDNA cloned by this method induces neuronal differentiation, and was obtained from a fetal (E17) rat hippocampal cDNA library. Expression of a truncated form of this cDNA turned on the expression of the full length endogenous ES cell gene. The DNA sequence for this gene showed no homology to any other sequences in the Genbank database, and we are currently charaterizing this gene further to identify domains within the cDNA responsible for inducing neuronal differentiation.

796.16

$^{(125)}$I DOI BINDING IN SPINAL TRANSECTED RATS.
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Mid-thoracic transection eliminates descending input to the lumbar spinal cord, thereby denervating target neurons. Fetal neural tissue transplanted into the lesion site permits regeneration of the descending serotonergic axons into and sometimes through the transplant into the host tissue caudal to the transplant, and thus may be expected to reverse denervation supersensitivity. 5-HT agonists improve locomotor function in animals receiving transplants as neonates or as adults through actions directly or indirectly on denervated targets (Miya et al., this volume). We characterized the serotonergic receptors in the spinal cord of spinal transected and transplanted rats. For these studies mid-thoracic transection at T6-T7 is performed in rats on postnatal day 0-2 or at 8 weeks. Transplants of E14 embryonic spinal cord were placed at the transection site in one group of animals. A separate group of normal littermates is maintained as unoperated controls. The spinal cords were harvested 2 weeks to 8 weeks postsurgery and processed for receptor autoradiography. 5-HT_2 receptors were labeled using $^{(125)}$I DOI, in spinal cord rostral and caudal to the area of transection/transplantation. In adult transected rats, we observed high levels of specific binding in lamina IX of the ventral horn, lamina I of dorsal horn and in the IML. Lamina IX caudal to transection showed significantly more binding than that seen rostral to the transection. The behavioral effects of serotonergic agonists are therefore likely to be mediated by supersensitive serotonergic targets in the ventral horn.
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796.17

FETAL HIPPOCAMPAL GRAFTS IN KAINATE LESIONED HIPPOCAMPUS ESTABLISH SPECIFIC CONNECTIONS AND REDUCE ABERRANT MOSSY FIBER SPROUTING.
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Lesion-induced host factors, particularly trophic support and denervation, dramatically enhance cell survival in micrografts transplanted to adult rat hippocampus at 4 days post-Kainic acid (KA) lesion [Shetty and Turner, Neuroscience]. We hypothesize that restoration of hippocampal throughput in the KA model critically depends on the establishment of specific graft-host connections. Large suspension grafts of E19 hippocampal cells were prelabeled with 5'-bromodeoxyuridine, and were transplanted at 4 days post-KA lesion. We have measured the formation of efferent connections after 3-4 months survival using Fluorogold and DiI tracing and serial section analysis. The effect of grafts on aberrant sprouting of mossy fibers into the dentate supragranular layer (DSGL) was also quantified in Timm's stained sections.

Many neurons in grafts located in the vicinity of the degenerated CA3 cell layer established commissural connections with the contralateral hippocampus (Mean±SEM=564±328, n=7). However, such connections did not occur with ectopic grafts (<5 cells per graft, n=5). Neurons within all grafts made connections with the medial septal nucleus (451±127, n=6). Host mossy fiber ingrowth into the graft area was denser for grafts located near the degenerated CA3 cell layer than other grafts within hippocampus. The region of aberrant mossy fiber sprouting in different blades of the DSGL (measured as both width and area per length) was significantly reduced in animals with grafts near the CA3 cell layer (n=5; $p<0.01$, 58-68% reduction) compared to animals with ectopic transplants (n=5).

These results clearly show that grafted fetal hippocampal neurons both partially reconstitute the damaged circuitry following KA lesions by establishing specific point-to-point connections with the host and significantly reduce the development of post-KA abnormal circuitry in hippocampus. Supported by RO1 NS29482-01 and VAMC.

796.18

TRANSPLANTATION OF SUBVENTRICULAR ZONE CELLS FROM ADULT MICE INTO THE NEOCORTEX AND STRIATUM.
Daniel G. Herrera and Arturo Alvarez-Buylla*. Cornell Medical Center and Rockefeller University.

The subventricular zone (SVZ) of adult mice contains neuronal precursors (Lois, C. & Alvarez-Buylla, A. *Proc. Natl. Acad. Sci. USA* 90, 2074-2077 (1993), Morshead, et al. *Neuron* 13, 1071-1082 (1994)). These cells are known to migrate along a well defined pathway into the olfactory bulb where they differentiate into granular and periglomerular neurons (Lois, C. & Alvarez-Buylla, A. *Science* 264, 1145-1148 (1994)). To date there is no evidence that these cells can form new neurons outside of the olfactory bulb *in vivo*. To test whether these precursors could form neurons when grafted into neocortex and striatum, we transplanted SVZ cells from adult transgenic mice carrying the ß-Galactosidase gene attached to the neuron specific enolase (NSE) promoter (Forss-Petter, S., et al. *Neuron* 5, 187-197 (1990)). The recipients, were adult non-transgenic mice that had undergone an ischemic cortical lesion or a kainic acid-induced lesion in the striatum 1 week prior to transplantation. The SVZ was microdissected, the cells were dissociated and 30,000 to 50,000 cells were transplanted into the lesion site of the recipient mice. The animals were allowed to survive for 6 weeks. The brains were processed histochemicaly to reveal the presence of ß-galactosidase. ß-gal+ cells were present in both striatal and cortical grafts. In 2/3 striatal lesions we counted 78 and 120 ß-gal+ cells. In the ischemic lesions in cortex between 2 and 17 ß-gal+ cells were observed in each graft. These results indicate that SVZ cells from the adult brain survive and can differentiate into neurons after transplantation.

796.19

EGF-responsive neural progenitor cells survive, migrate and differentiate after transplantation into the adult rat striatum.

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Neural progenitors or stem cells derived from the developing rat and mouse brain with the capacity to generate both neurons and glia can be grown in culture. The aim of our study was to characterize the migrational patterns and cell differentiation of neural progenitor cells after transplantation into the striatum.

Striata of E15-transgenic mouse embryos, carrying the lac-Z reporter gene under control of the glial fibrillary acidic protein (GFAP) promoter, were triturated to a single cell suspension and cultured in serum-free medium containing 20 ng/ml Epidermal Growth Factor. The arising so called neurospheres were triturated to a single cell suspension and re-seeded every week for 14 weeks. During the last week of culture the cells were labelled with ^{3}H-thymidine and about 500.000 cells were transplanted into the striatum of intact adult Sprague-Dawley rats. The animals were immunosuppressed with cyclosporin A. Survival time was two weeks.

Autoradiography revealed good cell survival: While about 25% of the labelled cells stayed in the transplantation core, about 75% migrated throughout the whole striatum, many cells also entering the white matter tracts of the corpus callosum and the internal capsule. Cells in the core of the transplant mainly differentiated into astrocytes as revealed by immunohistochemistry for ß-galactosidase (ß-gal) and GFAP in combination with autoradiography and by the mouse specific astrocytic marker M2.

However, some cells developed a neuronal phenotype as seen by staining with the mouse specific M6-antibody. The cells which had migrated away from the core were ß-gal negative and only a few cells stained for GFAP. Experiments determining the identity of the migrated cells are currently under investigation.

AGING PROCESSES: MOLECULAR CHARACTERISTICS

797.1

THE EFFECT OF AGE AND GONADAL STEROIDS ON THE EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN IN THE CEREBELLUM. J. E. Anderson, B. C. Jones*, J. P. O'Callaghan, J. R. Day. Depts. Of Biology and Biobehavioral Health, The Pennsylvania State Univ. University Park, PA 16802 and the Neurotoxicology Div., Health Effects Lab., U.S. E.P.A., Research Triangle Park, NC 27711.

Glial fibrillary acidic protein (GFAP) is an astrocyte-specific intermediate filament protein which is used as an index of reactive gliosis and neurodegeneration. GFAP increases in response to brain injury and normal aging, and its expression can be manipulated by altering circulating adrenal and gonadal steroids. Gonadal steroids decrease in women at the age of menopause, and testosterone concentrations are often reduced in older men. One hypothesis is that lower levels of gonadal steroids might render the brain more susceptible to neurodegeneration. The purpose of this study is to examine the effects of gonadal steroid manipulation on GFAP in the rat brain during aging. Three age groups (3, 12, or 24 mo) of male, Fisher 344 rats were castrated and given hippocampal lesions. Castrated animals received either testosterone implants or blank implants, resulting in marked differences in the concentration of circulating testosterone. In this preliminary study, GFAP immunoreactivity in the cerebellum (a region not effected by the lesion) was measured by ELISA and compared between age and hormone treatment groups. The results indicated that 3 and 12 mo intact and castrated animals had lower GFAP content than their respective 24 mo groups. Testosterone implants in castrated rats reversed this age-related increase in GFAP immunoreactivity. These data show that the exogenous testosterone suppressed the age-related increase in GFAP in the cerebellum, a brain region not generally associated with steroid hormone sensitivity. These data support the hypothesis that changes in gonadal steroid hormones during aging might render the brain more susceptible to neurodegeneration. Also, these data suggest that hormone replacement therapy might have value in neurodegenerative disease intervention. This study was funded by an AFAR research grant to JRD.

797.2

AGE-RELATED INCREASE IN TGF-ß1 mRNA IN RAT HIPPOCAMPUS IS LOCALIZED TO ACTIVATED MICROGLIA. N.R. Nichols*, C.E. Finch and T.E. Morgan. Andrus Gerontology Center, Dept. of Biological Sciences, Univ. Southern Calif., Los Angeles CA 90089-0191.

Increased TGF-ß1 expression occurs in adult brain in response to injury and disease and may mediate neurotrophic effects of glia. Previously, we showed by RNA blot hybridization that TGF-ß1 mRNA was up regulated in rat and human brain with advanced age. We also showed that TGF-ß1 was up regulated after lesioning of rat brain and was localized to activated microglia following both deafferenting and neurotoxic lesions. Several reports indicated that microglial numbers increased during aging and that microglia in the aged brain exhibited an activated morphology (shortened, thickened processes and increased expression of complement receptor 3 and MHC class II antigen). Collectively, these data suggested that the age-related increase in TGF-ß1 mRNA could be due to the increased number of microglia or to increased expression per cell. Therefore, TGF-ß1 mRNA was quantified in the hippocampus and cortex on a per cell basis in young (7 mo) and old (24 mo) F344 male rats following in situ hybridization with a [35]S-labeled cRNA probe. TGF-ß1 grain density (grains/cell) increased 59% ($P < 0.01$) in hippocampus and 55% ($P < 0.05$) in cortex of old compared with young rats. We also performed combined immunocytochemistry with antibodies against glial markers and in situ hybridization for TGF-ß1 mRNA. TGF-ß1 mRNA in old rats is predominantly localized to OX-42+ microglia with increased expression of complement receptor 3 compared with young rats. In contrast, TGF-ß1 mRNA in old rats did not co-localize with GFAP+ astrocytes. These data indicate that increased TGF-ß1 mRNA prevalence is a marker of activated microglia in the aged hippocampus and cortex. If increased TGF-ß1 peptide is secreted from activated microglia, this peptide may contribute to the neurotrophic activity of microglia in the brain during aging. Supported by AG-07909 (CEF) and AFAR (TEM).

797.3

TRANSFORMING GROWTH FACTOR (TGF)-ß1 AND THE MICROGIAL RESPONSE TO AGING. T.E. Morgan*, I. Rozovsky, T.H. Hogan, and C.E. Finch. Andrus Gerontology Center, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

Unlike microglia in young adult mammalian brain, microglia in aged brains appear to be in an activated state that we hypothesize may contribute to the age-related susceptibility to neurodegeneration through altered response to TGF-ß1. TGF-ß1 is a pleiotrophic peptide that increases during the brain's response to neurodegeneration and aging. In cultured microglia from neonatal rats, TGF-ß1 effectively suppresses activation, proliferation, and major histocompatibility factor class 2 expression. These studies examine the effects of TGF-ß1 on cultured microglia from aged rat brains. As observed in vivo, cultured microglia from 24 month old rat brain ("aged") are morphologically distinct from their "young" (3 month old) counterparts and appear to be in an activated state. The mitotic index (MI), calculated from ^{3}H-thymidine labeling, of "aged" cultured microglia is 4 times higher than the MI of "young" cultured microglia. TGF-ß1 (1 ng/ml, 24 hours) treatment does not inhibit the increased proliferation of "aged" cultured microglia. Furthermore, TGF-ß1 (1 ng/ml, 24 hours) failed to inhibit the lipopolysaccharide (LPS)-induced formation of reactive nitrogen intermediates (nitrite concentration in medium) in "aged" cultured microglia. Therefore, "aged" microglia are less responsive to the deactivating effects of TGF-ß1, which may be a factor in age-related diseases, such as Alzheimers disease. Supported by AG-07909 (CEF) and AFAR (TEM).

797.4

FOCAL SITES OF DEMYELINATION AND REMYELINATION (MICROPLAQUES) IN PERIPHERAL NERVES OF AGED CATS. C. Bertolotto*, J.K. Engelhardt, F.R. Morales and M.H. Chase. Department of Physiology, Department of Anatomy and Cell Biology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Previous morphological studies in our laboratory have demonstrated that segmental demyelination and remyelination are present in the peripheral nerves of old cats (Bertolotto C. et al. Soc. Neurosci. Abstr. Vol. 20; part 2, p. 1710, 1994). In the process of examing myelin alterations during aging, we found that demyelination and remyelination exhibited a consistent pattern along the fibers of peripheral nerves of old cats, which is the subject of this abstract.

Two adult cats (1 to 3 years-old) and three old cats (17 to 19 years-old) were used in this study. These animals were given a lethal dose of sodium pentobarbital and perfused with a saline solution and a fixative (2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4). Teased fiber preparations of the hindlimb nerves were stored in 100% glycerin. The fibers were mounted in pure glycerin and examined with a light microscope.

Teased myelinated fibers from the adult cats exhibited a smooth myelin surface with intact nodes of Ranvier. The internodes of each individual fiber were of similar thickness and length. The teased fiber preparations from the old cats exhibited regions that contained clusters of two or more adjacent fibers where demyelination or remyelination had occurred. These regions were delimited by apparently normal myelin. We refer to these regions, that appeared as small patches of abnormal myelin, as "microplaques".

These focal abnormalities in adjacent myelinated fibers in the peripheral nerves, to our knowledge, have never been described before in aging animals or in peripheral neuropathies. However, we believe they were present in previous studies of others because published microphotographs exhibit the same phenomena that we have described in the present report. The presence of microplaques suggests that, during aging, there is a focal process that affects clusters of Schwann cells which are located in close proximity to each other. *Supported by USPHS Grant AG 04307.*

797.5

DISTRIBUTION OF APOLIPOPROTEIN E PLAQUES AND GLIOSIS IN THE AGING RHESUS MACAQUE TEMPORAL CORTEX. S.G. Kohama* and H.F. Urbanski. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, Oregon, 97006.

Expression of the astrocyte marker, glial fibrillary acidic protein (GFAP) increases during aging and neuropathology. Similarly, the expression of apolipoprotein E (ApoE) increases with age in nonhuman primates in association with amyloid deposits. Because of the potential role of both of these markers in Alzheimer's Disease, the relative distributions of GFAP and ApoE were examined in the temporal cortex of the aging Rhesus macaque (*Macaca mulatta*).

The distribution of GFAP in the temporal cortex of young and middle-age adult monkeys was similar, with high levels seen in the molecular layers of the dentate granule cells, Ammon's horn and glial limitans. In some older monkeys (>25 years), hypertrophic astrocytes could be visualized in the hippocampal pyramidal and deeper layers of the temporal cortex.

ApoE expression was only seen in the older monkeys. Although a few plaques were seen scattered in the hippocampus of several of these animals, the majority of plaques were seen in the temporal cortex. Thus the more generalized distribution pattern of reactive astrocytes overlaps with that of the ApoE plaques, suggesting that a causal relationship may exist between astrogliosis and ApoE deposition.

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797.6

ALTERATION OF MULTIPLE GENE EXPRESSION IN INDIVIDUAL HIPPOCAMPAL CA1 NEURONS AS A FUNCTION OF AGING. Y. Cao* and J. H. Eberwine. Depts. of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Aging is recognized as a multifactoral event. The study of the interactions between these factors is essential to understand the fundamental mechanisms of the aging process. Using the antisense RNA amplification (aRNA) technique, we were able to simultaneously analyze multiple gene expression in individual cells in live acutely cultured slices of the hippocampal CA1 field. 3-month-old, 12-month-old and 24-month-old of Fischer 344 and Brown Norway F1 hybrid rats were used in the present study. We found that the mRNA levels for individual subunits of the glutamate receptor family were altered; specifically GluR2, GluR5 and GluR7 mRNAs decreased as a fraction of total composition of these receptor subunits as a function of age while GluR1, GluR3, GluR4 and GluR6 mRNAs had no significant compositional changes. Superoxide dismutase (SOD) mRNA also showed age-related changes, and interestingly the type II glucocorticoid receptor mRNA had higher expression in some but not all neurons from aged hippocampal slices. Presuming that protein levels parallel mRNA levels, then it is reasonable to postulate that coordinate changes in gene expression are reflective of functional significance.

797.7

AGE-ASSOCIATED CHANGES IN CNS TRANSCRIPTION FACTOR ACTIVITY. T. Toliver*, J. Papaconstantinou and R. Perez-Polo. Dept. of Human Biological Chemistry and Genetics, Univ. of Texas Medical Branch, Galveston, Texas 77555-0652.

Age-related cognitive deficits are usually associated with a loss of cholinergic function. Transcription factors may play an integral role in regulating cholinergic homeostatic gene expression. In the present study, we tested the hypothesis that transcription factor nuclear factor κB (NFκB) activity is altered in the basal forebrains and hippocampi of aged rats. Nuclear extracts prepared from basal forebrains and hippocampi of 3 and 30-month old Fischer-344 Brown Norway rats were used to measure NFκB activity by electrophoretic mobility shift assay. Basal levels of NFκB binding to cognate DNA consensus sequences were significantly higher in hippocampi ($p \leq 0.01$) and basal forebrains ($p < 0.05$) of aged rats. Basal AP-1 activity was also measured and showed no significant difference between age groups. NFκB activity in cerebellum and frontal cortex was also measured and there appear to be no significant differences between age groups. The data demonstrate increased basal levels of NFκB activity in the basal forebrain and hippocampus of the aged rat. The regional differences suggest a possible relationship between altered NFκB activity and decreased neurotrophin action and cholinergic function associated with aging. Supported in part by NINDS NS18708. This is publication #33A and is supported by USPHS grant P01AG10514 awarded by NIA.

797.8

AGEING-RELATED DECREASE IN PSA-NCAM IS NOT RELATED TO COGNITIVE DEFICITS. D.N. Abrous*, M.F. Montaron, [1] K.G. Petry, [2] G. Rougon, M. Darnaudéry, M. Le Moal, H. Simon, and W. Mayo. [1] INSERM U259, Bordeaux, France. [2] INSERM U394, Bordeaux, France. [2] CNRS-UMR 9943, Marseille, France.

Age-dependent spatial memory impairments have been related to a decline in hippocampal plasticity. It has been shown that highly polysialylated neuronal cell adhesion molecule (PSA-NCAM) are highly expressed during adulthood within regions associated with ongoing neurogenesis such as the hippocampus. Furthermore, NCAM defcient mice lack PSA-NCAM and show deficits in spatial learning when tested in the Morris water maze. The aim of the present study was to examine the effect of ageing on the expression PSA-NCAM within the hippocampus and other various brain regions using immunohistochemistry. In addition in order to investigate whether age-dependent changes in expression of PSA-NCAM were accentuated in aged rats with learning impairment, animals were in a first step assessed for their memory capability using a Morris water maze. Seven-month old and 24-month old rats were tested in the Morris water maze. Three weeks later animals were sacrificed and sections were processed for PSA-NCAM immunohistochemistry. Ageing was accompanied by an overall decrease in PSA-NCAM-immunoreactivity (IR) within the forebrain with a dramatic decrease of the number of PSA-NCAM-IR perikarya within the hippocampus and the piriform cortex. These results were confirmed by western blot analysis. No difference was observed in aged rats with or without spatial learning impairment. It is concluded that PSA-NCAM expression is dramatically reduced during ageing, very likely reflecting a decrease in neurogenesis. Although these PSA-NCAM changes parallel the decrease in cognitive abilities, our data did not evidenced a causal relationship between these two parameters.

797.9

BDNF AND SOMATOSTATIN GENE EXPRESSION IN THE PRIMATE BRAIN: DECREASED LEVELS OF mRNA DURING AGING. M. HAYASHI* and K. SHIMIZU. Department of Cellular and Molecular Biology, Primate Research Institute of Kyoto University, Inuyama, Aichi 484, Japan.

BDNF (Brain Derived Neurotrophic Factor) is one of the neurotrophic molecules for the various neurons in the vertebrate central nervous system. A recent study showed a marked decrease of BDNF gene expression in the hippocampus in Alzheimer's disease. Furthermore, in the rat cerebral cortex, BDNF was reported to enhance the level of mRNA of the neuropeptide somatostatin, suggesting that BDNF may be a regulatory molecule for the expression of the somatostatin gene. In the present study, using the northern blot analysis, we investigated BDNF and somatostatin mRNA expression in the central nervous system of the macaque monkey (*Macaca fuscata fuscata*) during the aging process. BDNF and somatostatin mRNA (1.6 and 4.0 kb transcripts for BDNF, 0.65 kb transcript for somatostatin) were detected in various cerebral subdivisions (the frontal cortex, the temporal cortex, the motor cortex, the somatosensory cortex and the visual cortex) and the hippocampus. During the aging process (2 years, 10 years and >30 years), the levels of BDNF and somatostatin mRNA significantly decreased in the cerebral subdivisions and the hippocampus. These findings suggest that the decrease in the gene expression of BDNF may cause the levels of somatostatin mRNA to decline in the primate brain during the aging process. Supported by Grant-in-Aid (04268103) from the Ministry of Education, Science and Culture, Japan.

797.10

CALCIUM BINDING PROTEINS AND SYNAPTIC VESICLE PROTEINS IN AGING RETINA. T.Yamaguchi*[1], P.Papazafiri[2], P.Podini [2]and J.Meldolesi[2]. [1]Dept. of Biochem. Tokyo Women's Medical Coll.,Tokyo Japan and [2] DIBIT, San Raffaele Scientific Institute , Milano Italy.

The changes of calcium homeostasis have been noticed to be one of an important aspect responsible for morphological and molecular deterioration in aging neurons. The functional alteration in aging retina has not yet been investigated from the point of calcium homeostasis. A high affinity calcium binding protein ,calretinin has been identified from retina which is densely distributed in sensory neurons besides retina and various subsets of cortical neurons was detected in the aging retina from 2 weeks to 30 months old rat. These changes were compared to that of another family member of calcium binding protein ,calbindin D28K ,in the retina as well as in the visual cortex. The synaptic vesicle proteins , synapsin and synaptophysin were also detected in the retina and cortex as marker proteins of synaptic function in aging neurons. Both synaptic marker proteins showed an age-dependent decrease only in the retina while calretinin ,densely distributed in the inner plexiform layer did not show any change in aging retina . Howerver, calbindin D28K, localized at the outer plexiform layer showed a comparable change in synaptic marker proteins in the retina. A dramatic decrease of synapsin at the inner plexiform layer which started after 12 month old was the most prominent change in aging retina,while in the cortices including occipital cortex,no decrease was observed. As a whole, age-dependent decrease is evident in synaptic marker proteins and calbindinD28K but not calretinin in the retina .On the contrary, in the cortex, none of these molecules showed age-depedent change.The role of these changes in aging retina remains to be disclosed.

797.11

INCREASING AGE ALTERS TRANSBILAYER FLUIDITY AND CHOLESTEROL ASYMMETRY IN SYNAPTIC PLASMA MEMBRANES OF MICE. U. Igbavboa, N. A. Avdulov, F. Schroeder, and W. G. Wood*. VA Med Ctr, GRECC and Univ of MN, Dept. of Pharmacology, Minneapolis, MN 55417.

Previous studies on age differences in membrane structure have reported on the total or average change in membrane structure. The present experiments determined fluidity and cholesterol distribution of the exofacial and cytofacial leaflets of brain synaptic plasma membranes (SPM) from 4-5, 14-15, and 24-25 mo old C57BL/6NNia mice using trinitrobenzenesulfonic acid quenching techniques and fluorescent probes. The exofacial leaflet of SPM from young mice was significantly more fluid as compared to the cytofacial leaflet. The large difference in fluidity between the two leaflets was abolished in SPM of the oldest age group. Age differences in leaflet fluidity were related to the distribution of cholesterol in the two leaflets. The exofacial leaflet contained substantially less cholesterol than the cytofacial leaflet (13% vs 87% respectively) in SPM of young mice. This asymmetric distribution of cholesterol was significantly modified with increasing age. There was approximately a two-fold increase in exofacial leaflet cholesterol in the oldest group when compared with the youngest age group. The bulk SPM cholesterol/phospholipid molar ratio did not differ among the three age groups. Transbilayer fluidity and cholesterol asymmetry were altered in SPM of older mice. This approach is a new and different way of viewing how aging modifies membrane structure. Age differences in SPM leaflet structure may contribute to modification of membrane function. Supported in part by AG11056 and Dept. of Veterans Affairs.

797.12

DISTRIBUTION OF OMP mRNA IN THE CILIARY-DENDRITIC, SOMAL AND AXONAL COMPARTMENTS OF HUMAN OLFACTORY RECEPTOR NEURONS. T.V. Getchell[*1], N.S. Rama Krishna[2], O.I. Buiakova[3], F.L. Margolis[3] and M.L. Getchell[2]. Depts. of [1]Physiology and [2]ENT Surgery, Univ. of Kentucky College of Medicine., Lexington, KY 40536, and [3]Roche Institute of Molecular Biology, Nutley, NJ 07110.

Differential localization of mRNAs in subcellular compartments may indicate a specific role for extrasomal protein synthesis in neuronal function. The localization of OMP mRNA and protein was examined in olfactory receptor neurons in tissue obtained at autopsy from humans who ranged in age from 26 weeks of gestation to 85 years of age, including three subjects with Alzheimer's disease. Quantitative *in situ* hybridization was performed on 10 µm-thick sections using ^{35}S-labeled antisense cRNA probes as described previously (Buiakova et al., *Genomics* 20:452, 1994; Rama Krishna et al., *NeuroReport* 6:817, 1995). The mean grain density was 1.7 X greater in the ciliary-dendritic, 4.0 X greater in the somal and 2.6 X greater in the axonal compartments than in the background. Also, the olfactory nerve layer-glomerular compartment in the olfactory bulb had a substantially higher grain density than tissue background. The mean grain density in the somal compartment increased systematically with age except in subjects with Alzheimer's disease where it was significantly less than in age-matched controls. The localization of grains over the compartments corresponded to the presence of mature olfactory receptor neurons as determined by OMP immunoreactivity at all ages studied. Our results indicate that OMP mRNA is localized in the ciliary-dendritic, somal and axonal compartments of human olfactory receptor neurons and that OMP mRNA is substantially reduced in subjects with Alzheimer's disease.

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797.13

EXPRESSION OF HEAT SHOCK PROTEINS IN AGING. M.C. Wilson, S.B. Maggirwar, T.J-F. Lee* and V. Ramkumar. Dept. of Pharmacology, Southern Illinois Univ. Sch. Med., Springfield, IL 62702

The expression of heat shock proteins by the cell helps it to cope with rapid changes in its environment such as exposure elevated temperatures, oxidants and toxins. In this study, we have assessed the expression of two heat shock proteins (hsp), a constitutive form (hsp70) and an inducible form (hsp72), in the brains of adult (3 month old) and aged (18, 24 month old) rats. The levels of hsp70, quantitated by Western blotting, in the 18 and 24 month old rats were 92 ± 8% and 83 ± 20% (mean ± SE) of the 3 month old, respectively. The basal levels of hsp72 for the 18 and 24 month old animals were 103 ± 3 and 124 ± 8% of 3 month old, respectively. Exposure of these different groups of rats to heat stress (37°C, 1 h) resulted in substantial age-related differences in the induction of hsp72 following 8 h of recovery. Increases in hsp72 elicited by heat stress were 815 ± 17%, 202 ± 14% and 101 ± 8% compared to the naive 3, 18 and 24 month old control, respectively. Increases in hsp70 were negligible under identical conditions, being 109 ± 6%, 100 ± 9% and 107 ± 6% of the respective naive age-matched controls. These studies demonstrate that the aging brain has a markedly reduced response to stress, which might make it more susceptible to damage following adverse changes in its local environment.

797.14

● THE DISTRIBUTION OF UBIQUITIN-PROTEIN CONJUGATES IN VARIOUS REGIONS OF RAT BRAINS ● E. R. Mesco*
Department of Biology, Moorhead State University, Moorhead, Minnesota 56563

The accumulation of varied molecular compounds is seen during the course of normal aging. In addition, specific lesions are associated with various age-related neuropathologies. These age-associated accumulations may, in part, be the result of alterations in proteolytic processing. The ATP-dependent ubiquitin proteolytic system is one candidate for such changes. Studies were performed using young (1 to 3 months old) Sprague-Dawley rats, both male and female. Brain tissue was isolated from cortex, cerebellum, hippocampus, striatum, and thalamus. Analysis of proteins was done using SDS-PAGE and immmunological identification with anti-ubiquitin antibodies. Profiles from the different brain regions were determined in order to establish baselines for future comparison to aged brain samples.

797.15

CELLULAR STORES OF THE IRON- AND ALUMINUM-BINDING PROTEIN, L-CHAIN FERRITIN, ARE MASSIVELY ELEVATED IN OLD HIPPOCAMPUS. S.R. Robinson[1,2] D.F. Noone[1,2] and T.A. Day[2]*
[1]Vision, Touch & Hearing Research Centre; and [2]Department of Physiology and Pharmacology, University of Queensland, St. Lucia, QLD, 4072. Australia.

Neuronal damage and loss in the aging brain may be caused by chronic exposure to free radicals. The generation of free radicals is rate-limited by the availability of iron and aluminum, which are stored within cells by ferritin. We have compared the distribution and intensity of immunolabeling for ferritin in the hippocampus of adolescent (3 months; n=7) and old (>20 months; n=7) male rats. Rats were euthanased with sodium pentobarbitol (60mg/kg; IP), perfused transcardially with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.2), and their hippocampal formations sectioned at 100µm on a Vibrotome. Sections were incubated in L-chain ferritin antisera for 5 days (1:2500; ICN), then in secondary antisera and finally with streptavidin-HRP-DAB.

In adolescent rats, cells with weak ferritin immunoreactivity are common in the stratum radiatum of CA2, but not elsewhere. There are no significant differences between individual rats in the total numbers of labeled cells ($p>0.1$; 2-tailed t-test). In old rats, the hippocampus contains more L-chain ferritin than any other brain region. Large numbers of strongly labeled cells are found in the stratum radiatum of CA2, CA3, CA4 and, to a lesser extent, the subiculum. The total number of ferritin-immunoreactive cells in old hippocampus is 6.5-fold higher than in adolescent hippocampus ($p<0.0001$; 2-tailed t-test), with CA4 showing a 78-fold increase. CA1 is consistently devoid of labeled cells. The total number of labeled cells differs significantly between individual old rats ($p<0.05$; 2-tailed t-test). Comparisons with different titrations of the primary antisera indicate that the maximal intracellular concentration of ferritin in old hippocampus is an order of magnitude higher than in adolescent hippocampus. At both ages, most ferritin-immunoreactive cells resemble resting microglia. Microglia may be implicated in age-related hippocampal degeneration through their storage of iron and aluminum, or through an associated production of free radicals.

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797.16

DECREASE IN LIPOPIGMENTATION IN HEARTH AND SYMPATHETIC GANGLION BY NITROCATECHOLS NITECAPONE AND ENTACAPONE. A. Hervonen, S. Lahtivirta, O. Kajander, M. Savola*, L. Sopanen# and P. Pohto#. Lab. of Gerontol., Univ. of Tampere, 33101 Tampere, Finland and # Orion Corporation, Orion-Farmos Pharmaceuticals, Helsinki, Finland.

Nitecapone and entacapone are new COMT-inhibitors with antioxidant properties. We studied lipopigmentation after two year administration of nitecapone and entacapone to Spraque-Dawley rats. Intragastric administration of nitrocatechols was used as a basic method of a toxicological study. Both male and female rats and three different doses were used. Left ventricle of hearth (LV) and superior cervical sympathetic ganglion (SCG) have been analyzed by now. The tissues were freeze-dried, fixed with paraformaldehyde vapor and embedded in paraffin. Lipopigmentation was measured using image analysis system connected to a fluorescence microscope. Both nitecapone and entacapone diminished the area covered by lipopigments both in SCG and LV. The pigment granule size was smaller in nitrocatechol groups. This decrease was more drastic in females and especially with entacapone. The best results with low lipopigmentation were obtained with intermediate doses (80-90 mg/kg) of nitrocatechols. In female intermediate groups the area covered by lipopigments was about 50% of the control value. This may be due to the antioxidative properties of nitecapone and entacapone. The compounds may provide protection against oxidative stress in a lifelong experiment.

797.17

EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) IN SPINAL MOTOR NEURONS AFTER AXOTOMY AND IN AGING F344 RATS. **O. Hanson-Painton*, P. Grammas and J.M. Jacob. Depts. of Pathology and Anat. Sci., Univ. of Oklahoma HSC, OKC, OK 73190.**

Nitric oxide (NO) has been shown to be both an important neuromodulator and neurotoxin in the CNS. The objective of this study was to examine the effects of age and axotomy on iNOS expression in spinal motor neurons. In 6 month F344 rats, the sciatic nerve was cut and iNOS expression examined at 1-7 days after axotomy. The retrograde tracer Fluorogold was used to locate the motor columns supplying the right sciatic nerve. Using a polyclonal antibody directed against iNOS and avidin-biotin enhanced DAB to visualize protein immunoreactivity (IR), the distribution and temporal expression of iNOS-IR was determined. The data demonstrate iNOS-IR was increased by day 2 and maximally upregulated by day 3; by day 7, iNOS-IR was reduced but still above control levels. Also, a comparison of 6 and 24 month spinal motor neurons showed a marked increase in iNOS-IR. These data demonstrate an upregulation of iNOS after axotomy and in aging, suggesting increased production of NO with axonal injury and age. Supported by grants to PG (NS 30457) and JMJ (AFAR).

797.18

AGE-DEPENDENT INCREASE IN INDUCIBLE NITRIC OXIDE SYNTHASE IN CEREBRAL MICROVESSELS. **M.A. Dorheim*, W.R. Tracey and P. Grammas. Dept. of Pathology, Univ. of Oklahoma HSC, Oklahoma City OK 73104, Dept. of Cardiovascular and Metabolic Diseases, Central Research Division, Pfizer Inc., Groton CT 06340**

Vascular endothelial cells are a quantitatively important producer of nitric oxide (NO) and could be a source of this mediator/neurotoxin in the brain. We have previously demonstrated that isolated rat brain microvessels possess both constitutive (ecNOS) and inducible nitric oxide synthase (iNOS). The objective of this study was to compare iNOS levels in microvessels from 3 month-old adult rats to that present in microvessels isolated from aged (18-23 months) animals. Intact microvessels were isolated from rat cerebral cortices, homogenized and supernatants collected. Immunodetection was performed using an anti- iNOS antibody (#8196, 1:1000) and a peroxidase labelled secondary antibody. Slot blot quantitation was performed by scanning densitometry. iNOS protein was found to be >12-fold higher in the microvessels from the aged rats compared to the 3 month-old rats. These data suggest that increased expression of iNOS and a concomitant elevation of NO production may contribute to neuronal damage in the aged brain. (Supported in part by NIH NS30457 and OCAST)

CHLORIDE AND OTHER CHANNELS

798.1

A CALCIUM-DEPENDENT CHLORIDE CURRENT IN MOUSE SYMPATHETIC NEURONS . F. de Castro, E. Geijo-Barrientos and R. Gallego*. Instituto de Neurociencias y Departamento de Fisiología, Universidad de Alicante, 03080-Alicante, Spain.

Axotomized rat sympathetic neurons show a depolarization after spike firing (ADP), which is generated by a calcium-dependent chloride current (Sánchez-Vives & Gallego, *J. Physiol.* 475: 391, 1994). In normal cells this conductance may be located in the dendrites and only become evident in somatic recordings after the dendritic retraction induced by axotomy. Therefore, we have investigated whether this current is present in non-axotomized mouse sympathetic neurons which have shorter dendrites than similar cells in the rat. The experiments were done in an "in vitro" preparation of the superior cervical ganglion using single-electrode current- and voltage-clamp techniques and intracellular staining with neurobiotin. In the presence of TTX (1 μM) and TEA (25 mM), inward peaks and outward currents were recorded during 50-200 ms depolarizing pulses from -50 mV, followed by slowly decaying inward tail currents that lasted 400-800 ms. The tail current was blocked by anthracene-9-carboxylic acid (2 mM), a selective blocker of chloride channels, and its reversal potential shifted in accordance with the Nernst equation when the extracellular chloride concentration was changed. Calcium-free solution or $CdCl_2$ (200 μM) abolished both, the inward peak and the tail current. In current-clamp recordings, a train of spikes evoked a marked ADP in the majority of the cells. The amplitude of the ADP for a given neuron was inversely correlated with the number and total length of dendritic branches. These results suggest that mouse sympathetic neurons have a calcium-dependent chloride current localized in the distal dendrites.

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798.2

SEROTONIN-ACTIVATED Cl^- CURRENT IN RAT BRAIN STEM NEURONS

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We studies RN46A cells, a serotonergic neuronal cell line derived from E13 rat raphe (White et al., J.Neurosci,1994). Intracellular recordings in the whole-cell configuration were made at room temperature using CsCl-filled micropipettes. 5-HT receptor agonists and antagonists were applied either by pressure pulses from closely positioned pipettes or by gradual diffusion in the extracellular medium. Applications of 5-HT evoked a dose-dependent inward current when holding membrane potentials were negative. When the equilibrium potential for Cl^- was set near 0 mV, current responses to 5-HT reversed polarity near 0 mV suggesting a dominant role for Cl^- ions in the conductance response. Bicuculline and strychnine had no effect on 5-HT activating current while picrotoxin blocked the Cl^- current. The slow kinetics of activation of the Cl^- conductance suggest the participation of second messengers in this process. (Supported VAMC MRIS #1769 and 3369 and USPHS #NS 17577).

798.3

GABA AND PENTOBARBITAL ACTIVATED CURRENT IN HUMAN DRG CELLS

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Cells were cultured from embryonic (E) day 72 and adult human dorsal root ganglia (DRG). Neurons were grown for 5 or more days in serum-supplemented medium. Intracellular recordings in the whole-cell configuration were made at room temperature. $GABA_A$ receptor agonists and modulators were applied by pressure pulses from closely positioned pipettes. All cells responded to micromolar concentrations of GABA and the general anesthetic pentobarbital, but not the steroid anesthetic alphaxalone (3α-hydroxy-5α-pregnane-11,20-dion). When the equilibrium potential for Cl^- was set near 0 mV, current responses to GABA and pentobarbital reversed polarity near 0 mV, which corresponds to E_{Cl^-} calculated from the Nernst equation and suggests a dominant role for Cl^- ions in the response. Currents activated by GABA and pentobarbital were blocked by bicuculline and picrotoxin. Remarkably, diazepam had no modulatory effect on GABA-activated current. Noise analysis of membrane current variance shows that the open times of Cl^- channels activated by GABA and pentobarbital are different. (Supported by VAMC MRIS #1769 and 3369 and USPHS #NS 17577).

798.4

COMPARISONS OF AND INTERACTIONS BETWEEN THREE TRANSMITTER-INDUCED CL-DEPENDENT RESPONSES IN *APLYSIA* NEURONS. J.S Kehoe* and C.A Vulfius. Lab. de Neurobiologie, Ecole Norm. Sup., 46 rue d'Ulm, 75005 Paris

King and Carpenter (Neurosci. Lett. 82:343-348, 1987) observed that transmitter-induced Cl-dependent responses in *Aplysia* neurons often "interact" with one another: they cross-desensitize, and are often blocked by the same antagonists. We have studied the Cl-dependent responses of the Medial cells of the pleural ganglion to fast perfusion application of ACh, GABA and Glutamate. None of these responses, studied in whole-cell patch clamp, could be selectively blocked by the antagonists tested (strychnine, picrotoxin, bicuculline, and tubocurarine). Furthermore, each of these transmitter-induced responses desensitized with prolonged agonist application, and a "desensitizing" pulse of any one of the three transmitters caused a significant cross-desensitization of the responses induced by the other two. Even when using ATP- GTP-free solutions in very low resistance whole-cell pipettes, all three responses, as well as their cross-desensitizing interactions, persist.

In spite of the marked interaction between the GABA response and those elicited by ACh and Glutamate, the GABA response appears to be mediated by a different channel. When sulphate was used as the anion in the pipette solution, the GABA response was reduced by about 70-80% whereas the responses to ACh and Glutamate remained unchanged. In contrast, when Cl was used, all three responses, measured at a fixed number of mV less negative than ECl, remained unchanged. It thus appears that the interactions between these transmitter-induced responses are not simply the result of a common activation of a single receptor-channel complex.

798.5

SELECTIVE ANION PERMEABILITIES DISTINGUISH SUBCELLULAR CALCIUM POOLS IN RAT BRAIN.

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The anion selectivity of rat brain intracellular, nonmitochondrial $^{45}Ca^{2+}$ sequestering compartments was studied using rat brain microsomal fractions and fresh frozen sections. Mg-ATP dependent $^{45}Ca^{2+}$ transport in sodium azide containing buffers showed a strict requirement for anion cotransport as no $^{45}Ca^{2+}$ uptake occurred in buffers containing the impermeant gluconate as the sole anion. Prominent $^{45}Ca^{2+}$ accumulation was observed with oxalate, fluoride and phosphate as the sole anions and intermediate accumulation using acetate or chloride. By using a number of Ca^{2+} transport inhibitors two distinct compartments could be distinguished, each showing unique anion preferences. Thapsigargin (Tg), cyclopiazonic acid (CPA) and 2,5-di-*tert*-butyl-hydroquinone (tBQ) potently blocked oxalate, fluoride,or phosphate supported Ca^{2+} transport. Inhibition of Mg-ATP dependent chloride supported $^{45}Ca^{2+}$ uptake, however, required much higher concentrations of these inhibitors. Autoradiographic localization of $^{45}Ca^{2+}$ sequestering compartments using fresh frozen rat brain sections revealed unique anatomical distributions for the chloride selective pool with enrichment in brain stem, deep cerebellar nuclei and spinal cord. $^{45}Ca^{2+}$ uptake supported by oxalate, fluoride or phosphate was instead much more prominent in forebrain structures and cerebellar cortex.

798.6

RINGER CONDITIONS DETERMINE THE STABLE EXPRESSION OF WHOLE-CELL CHLORIDE CURRENT IN RAT ASTROCYTES.

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Increases in $[Cl^-]_i$ accompany astroglial depolarization in intact slices and primary cultures[1-2], suggesting a role for Cl^- in many activity-dependent processes. The dominant hypothesis based on whole-cell, excised and cell-attached patch clamp recordings, however, is that astroglial chloride channels are normally inhibited in culture by some constitutive intracellular factor[3].

Cultures of astrocytes were prepared from neonatal rat pups, and maintained in DMEM (10% FBS), following standard techniques. Whole-cell patch-clamp recordings were made at 22-25°C using 140mM NMDG-Cl bath and 40mM NMDG-Cl/ 100mM NMDG-gluconate pipette solutions, both of which also contained Mg^{2+}. Following a >1 hour incubation of cells at 37° C in standard Ringer solution (10 mM dextrose; 140mM NaCl), we observed whole-cell chloride currents in 83% of cells 2-20 days after plating (n=24). Cells were voltage-clamped from -100 mV to +100 mV in 20 mV increments to reveal outward rectifying chloride currents as expected in asymmetrical Cl^- solutions. The mean peak amplitude was 780 pA and the current reversal potential was -26.6 ± 9.4 mV (n=20). When gluconate was substituted for 100 mM Cl^-, the reversal potential was 8.6 ± 5.0 mV (n=5), and the current-voltage (I-V) relationship was linear, suggesting no voltage-dependence. When gluconate was substituted for all but 8 mM Cl^-, the reversal potential depolarized to 31 ± 5.1 mV (n=4), and the I-V became inwardly rectifying. Chloride currents were completely but reversably blocked by 1mM Zn^{2+} (n=3), a known chloride channel blocker. In cells that were introduced to recording solutions at 22-25°C after removal from DMEM, no whole-cell chloride current was observed (n=19).

These results demonstrate that removal of cells from serum-containing media and possibly temperature are important determinants of chloride current expression in cultured astrocytes. 1. Ballanyi et. al., *J. Physiol.*, **382**, 1987; 2. Walz & Mukerji, *Exp. Neurol.*, **99**, 1988; 3. Barres et. al., *Annu. Rev. Neurosci.*, **13**, 1990.

798.7

SWELLING-ACTIVATED AMINO ACID EFFLUX IN THE HUMAN NERUROBLASTOMA CELL LINE CHP-100.

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Pathological disturbances (such as hyponatermia or ischemia) may result in significant changes in neuronal cell volume which have not been investigated in detail. The present studies evaluated the effects of hypoosmotic stress on cell volume and amino acid efflux in the human neuroblastoma cell line CHP-100. Using a Coulter Multisizer, CHP-100 cells were found to swell by ~35% ± 5% when the osmolarity of the extracellular solution was decreased from 290 to190 mOsm/kg H_2O. The rapid swelling was followed by regulatory volume decrease (RVD) with the cell volume approaching the isoosmotic value over 15 min. In cells loaded with ^{14}C-taurine, a similar hypoosmotic shock increased taurine efflux to 299% ± 22% (n=23, $p<0.05$) of control values. This efflux was inhibited by the chloride channel blockers 5-nitro-2-(3-phenyl-propylamino)-benzoic acid (NPPB), 4,4'-diisothiocyanatostilbene-2,2'-disulfonic-acid (DIDS), and niflumic acid, and was also dependent upon extracellular calcium. Hypo-osmotic stress also caused a significant increase in ^{14}C-glycine efflux, that was sensitive to NPPB. By contrast, efflux of ^3H-glutamate was not significantly affected by hypoosmotic exposure. It is concluded that CHP-100 cells undergo RVD when confronted with hypoosmotic stress and efflux of taurine and glycine may contribute to this process via a calcium-dependent anion permeability pathway.

798.8

LITHIUM PRE-TREATMENT PREVENTS ACTIVATION OF VOLUME-SENSITIVE CHLORIDE CHANNELS IN BOVINE CHROMAFFIN CELLS.

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The role of phosphatidylinositol 4,5-bisphosphate (PIP2) hydrolysis in activation of volume-sensitive chloride (VolS Cl) channels was studied in bovine chromaffin cells using whole-cell patch clamp. Its involvement is implicated by inhibition of the VolS Cl channels by intracellular neomycin (Doroshenko & Neher, 1991) which is known to selectively bind PIP2 making it unavailable as a substrate for PIP2-specific enzymes (e.g. phospholipase C-γ). It has also been reported that hypotonic swelling of astrocytes intensified PIP2 hydrolysis, while its inhibition resulted in suppression of regulatory volume decrease (Bender *et al.*, 1993). The ability of bovine chromaffin cells grown in primary culture to generate the Cl current in response to intracellular administration of 100 μM GTPγS was tested during their incubation in the presence of 10 mM LiCl. Because LiCl is a potent inhibitor of inositol monophosphatase involved in replenishment of the limited membrane pool of PIP2, its presence is expected to cause a depletion of PIP2 in the cell membrane. From about 10 cells tested from each coverslip treated with LiCl, only the first cells tested within 10 min after the drug addition to the bath produced the current, while those tested after longer exposures did not. These results support the hypothesis that activation of VolS Cl channels in bovine chromaffin cells involves stimulation of PIP2 hydrolysis and suggest the role for lipid-derived second messengers in volume-regulatory processes.

798.9

MECHANISM OF ACTION OF FLUFENAMIC ACID ON Ca-ACTIVATED CHANNELS.

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Non-steroidal anti-inflammatory drugs (NSAIDs) are well known to produce anti-inflammatory effects through actions on prostaglandins, but their mechanism of analgesic action is less well understood. We have shown previously that the NSAID, flufenamate (FFA) transiently activates both a Ca-activated nonselective (I_{CAN}) and a Ca-activated outward ($I_{o\text{-}slow}$) current in neurons and that this is paralleled by a maintained rise in $[Ca]_i$. These effects are specific and not shared by two other fenamates.

In this study, we investigated the mechanism of action of FFA on $[Ca]_i$ and these two currents. A rise in $[Ca]_i$ is still observed following FFA application in dinitrophenol or thapsigargin but the transient increase in I_{CAN} and I_{slow} is reduced. Application of protein kinase inhibitors and membrane permeable cAMP analogs suggest that the effect on membrane currents is not through channel phosphorylation. Both the $[Ca]_i$ response and the current activation process were fully recovered after a 20 min wash following FFA application.

We conclude that FFA causes a release of Ca from mitochondria and ER that activates these Ca-activated currents and that these stores can be rapidly refilled. The reduction in I_{CAN} in the presence of a maintained elevation of $[Ca]_i$ does not appear to result from channel phosphorylation.

These are the first descriptions of the mechanism of action of this NSAID on neuronal Ca-activated channels. These actions may underlie some of the analgesic actions of this drug.

798.10

CALPAIN ACTIVATED CHLORIDE CHANNELS IN EXCISED PATCHES OF MYOTUBES AND NEURONS.

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We have previously reported that exogenous calpain activates chloride channels when applied to the cytoplasmic face of about half of excised patches from mouse myotubes. We now report a similar activation in cerebellar granule cells and provide evidence that a membrane-resident protease may be responsible for activating the channel when it arises spontaneously. The channel in both cell types is outwardly rectifying, has a conductance between 30 and 90 pS and is blocked by 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), similar to channels seen in cells from airway epithelium and several other tissues. Channels are also activated when bath calcium is raised from 0.18 to 1.8 mM, again in about half of excised myotube patches, and this effect is blocked by the presence of the protease inhibitor leupeptin. Cytochalasins have been reported to activate chloride channels in myoballs and other cells and we report here such an activation in neurons. This and the fact that cytoskeletal proteins are known to be calpain substrates suggest that proteolytic activation of the chloride channel may be mediated via a cytoskeletal regulation of the channel.

798.11

DEVELOPMENTAL EXPRESSION OF AN INWARDLY RECTIFYING CHLORIDE CONDUCTANCE IN RAT BRAIN. G.H. Clayton*, R.L. Smith, K.J. Staley, and C.L. Wilcox. Dept. of Neurology, UCHSC, Denver, CO 80262 and Dept. of Microbiology, CSU, Fort Collins, CO 80523.

A voltage sensitive, inwardly rectifying chloride channel, which has been shown to play a significant role in the modulation of neuronal responses to $GABA_A$ activation, is present in the same neurons that express mRNA for a previously cloned chloride channel (ClC-2) with similar properties. These results strongly suggest that ClC-2 is the source of the inwardly rectifying conductance. Using digoxigenin labeled riboprobes, we have examined the expression of ClC-2 during early postnatal development of rat brain and have found rapid changes in the pattern of developmental expression of ClC-2. In hippocampus and in cortex, expression is more wide spread in neonatal animals than in adult rats and can be found in proliferative layers. Similarly, ClC-2 was expressed in neurons of caudoputamen in the early stages of post natal development but was essentially absent in adult animals. In contrast, in cerebellum, the expression of ClC-2 is a relatively late post-natal event occurring between 7 and 14 days after birth during the period of purkinje cell synaptogenisis. To summarize, ClC-2 is expressed extensively in developing rat brain neurons including cells within germinal layers and becomes more restricted in the adult. These results suggest that ClC-2 may subserve functions which are different during migration and synaptogenesis than in the adult.

CALCIUM CHANNELS: MISCELLANEOUS BLOCKERS

799.1

NIMODIPINE INHIBITS A PERSISTENT CALCIUM CURRENT IN CHRONICALLY DEPOLARIZED CEREBELLAR GRANULE NEURONS C.Usai*, C.Marchetti, A.Barberis and L.Moccagatta. Istituto di Cibernetica e Biofisica, CNR, via De Marini, 6, 16149, Genova, Italy.

High $[K^+]_o$ in the culture medium is a standard technique for maintaining *in vitro* viability of neurons in defect of appropriate neurotrophic factors. Survival promotion by high $[K^+]_o$ is a long term phenomenon; cells, indeed, live for weeks in absence of trophic factors. This effect is thought to be mediated by a chronic membrane depolarization inducing a sustained elevation of free $[Ca^{++}]_i$. The nature of the voltage-dependent Ca^{++} channel responsible for this persistent elevation was investigated in cultured cerebellar granule neurons from 8-day old rats, by measurements of $[Ca^{++}]_i$ in Fura2-loaded cells. Cultures, routinely maintained in 25 mM KCl to enhance survival, were incubated with Fura2 either in 25 mM (chronic depolarization) or 5.4 mM KCl (control solution). In 25 mM KCl, $[Ca^{++}]_i$ was 155± 32 nM (n=13), and quickly decreased to 43±19 nM (n=9) when $[KCl]_o$ was lowered to 5.4 mM. Saturating doses of nimodipine (>10 nM) reduced $[Ca^{++}]_i$ to the *same level* as in control solution. The IC_{50} of this effect was 0.39±0.09 nM. In contrast, agatoxin IVA (200-500 nM) was ineffective. In 5.4 mM KCl the basal calcium level was 36±15 nM (n=95), and acute depolarization with 25 mM KCl induced a $[Ca^{++}]_i$ elevation larger (≈300 nM) than in chronic depolarization, suggesting the presence in the last case of channel inactivation. In neurons depolarized with 15-75 mM KCl, nimodipine strongly inhibited the $[Ca^{++}]_i$ rise. The dose-dependence of this effect was best approximated by a two site curve with $IC_{50}(1)$=0.27 nM and $IC_{50}(2)$=65 nM. In agreement with previous findings in different tissues, these values might represent the affinities of the drug for two different states of the channel, namely closed (or resting) and open state. Chronic depolarizations activate DHP-sensitive Ca^{++} channels: the high level of $[Ca^{++}]_i$ due to the Ca^{++} influx through these channels is somehow responsible for the enhanced survival of granule cells.

799.2

EFFECTS OF NEFIRACETAM AND OTHER NOOTROPICS ON NEURONAL CALCIUM CHANNELS AND PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS. S. Watabe*[1], Y. Nakamoto[2], M. Yoshii[2], and T. Shiotani[1]. [1]Tokyo R & D Center, Daiichi Pharmaceutical Co., Ltd., Tokyo 134 and [2]Dept. of Neurophysiology, Tokyo Institute of Psychiatry, Tokyo 156, Japan.

We have previously reported that neuronal Ca^{2+} channels were blocked by the peripheral-type benzodiazepine receptor (PBR) agonist Ro 5-4864, whereas they were activated by the nootropic nefiracetam (DM-9384), although this compound has a binding affinity to PBR. In the present study, we have further investigated the effects of nefiracetam on the Ca^{2+} channel and PBR by using various nootropic agents. Ca^{2+} channel currents were optimally enhanced by nefiracetam at 1 μM and less potently by aniracetam at 10 μM. The currents were not affected by oxiracetam or piracetam, or chemically-unrelated cognitive enhancers such as idebenone and bifemelane. Unlike nefiracetam and aniracetam, these compounds inhibited the currents at higher concentrations. Binding of ^{3}H-PK 11195, a PBR antagonist, to the mouse brain was inhibited by nefiracetam and aniracetam with IC_{50}'s of 0.39 and 1.59 mM, respectively, whereas it was not inhibited by oxiracetam and piracetam. The results suggest that PBR is a low-affinity site for nefiracetam and aniracetam and may be involved in their drug actions at high concentrations, whereas PBR is not involved in drug actions of other cognitive enhancers such as oxiracetam and piracetam.

799.3

FACILITATORY ACTION OF THE NOOTROPIC NEFIRACETAM (DM-9384) ON CALCIUM CHANNELS IS MEDIATED BY INHIBITORY G-PROTEINS IN NG108-15 CELLS. M. Yoshii*[1], S. Watabe[2], T. Nukada[3], T. Shiotani[2] and M. Tanaka[2]. [1]Dept. of Neurophysiology, [3]Dept. of Neurochemistry, Tokyo Institute of Psychiatry, Tokyo 156 and [2]Tokyo R & D Center, Daiichi Pharmaceutical Co., Ltd., Tokyo 134, Japan.

We have recently reported that the cognitive enhancer, nefiracetam (DM-9384), increases L-type Ca^{2+} channel currents in NG108-15 cells and that the effect is inhibited by pertussis toxin (PTX), suggesting that inhibitory G-proteins (Gi/Go) mediate the drug action (Yoshii and Watabe, *Brain Res.* **642**, 123-131, 1994). In the present study, we have further examined whether stimulatory G-proteins (Gs) are involved in the action of nefiracetam using the whole-cell patch-clamp technique. Under the condition that L-type currents were blocked by nifedipine (10 μM), the remaining high voltage-activated long-lasting currents were recorded. Leu-enkephalin (50 nM), which is known to activate Gi/Go, reduced the currents in a PTX-sensitive manner. The inhibited currents were reversed by nefiracetam (1 μM). On the other hand, PGE1 (10 μM), which is known to activate Gs, similarly reduced the currents, whereas dibutyryl cAMP (1 mM) did not affect the currents. The inhibited currents by PGE1 were not recovered by nefiracetam. The results suggest that the nefiracetam action is mediated by inhibitory G-proteins (such as Gi/Go), but not by stimulatory G-proteins (Gs).

799.4

LIFARIZINE BLOCKS VOLTAGE SENSITIVE NEURONAL CALCIUM CHANNELS. C.J. Siok,* R.D. Williams, L.F. Lanyon, R.J. Post, D. Yohannes M.K. Ahlijanian and A.H. Ganong Pfizer Inc, Central Research Division, Groton, CT 06340.

Lifarizine (RS 87476) is a use- and voltage-dependent sodium channel blocker (*Br. J. Pharmacol.*, 113:600, 1994) which also blocks the effects of calcium channel activators in smooth muscle (*Br. J. Pharmacol.*, 104:156P, 1991) and is neuroprotective (*Radiology*, 179:221, 1991). We examined the effects of lifarizine on several classes of neuronal calcium channel in both rat and human tissue. In rat SCG cells, using a holding potential of -100 mV, 10 μM lifarizine blocked 70±2% (n=3) of the nifedipine resistant current while 1 μM blocked 25±1% (n=6). The N-channel blocker conotoxin GVIA blocks approximately 70% under the same conditions. When the holding potential was lowered to -50 mV, the block by 1 μM lifarizine significantly increased to 47±4% (n=6; p<.001). Against P-type calcium channels in acutely dissociated rat cerebellar Purkinje cells 10 μM lifarizine blocked 52±5% (n=3) of the ω-Aga-IVA-sensitive current at a holding potential of -80 mV. The effects of lifarizine on human calcium channels were tested using fura-2 fluorescence measurements in the human IMR32 neuroblastoma cell line. Lifarizine blocked the ω-conotoxin GVIA-sensitive (N-channel) KCl-induced calcium increase with an IC_{50}=3.4 μM and nifedipine-sensitive (L-channel) increase with an IC_{50}=2.8 μM. At 10 μM, lifarizine also blocked 98% the current remaining in these cells after treatment with saturating concentrations of both nifedipine and GVIA. These data indicate lifarizine is a non-specific, voltage dependent blocker of neuronal calcium channels which, along with its ability to block voltage sensitive sodium channels, may play an important role in its neuroprotective activity.

799.5

VERAPAMIL AND LIDOCAINE PRODUCE A VOLTAGE-DEPENDENT BLOCK OF CLASS B CALCIUM CHANNELS EXPRESSED IN XENOPUS OOCYTES. M.L. WEBER*[1], G.W. CAMPBELL[1], C.P. TAYLOR[1], J. OFFORD[2] AND D.M. ROCK[1] [1]Neuroscience Therapeutics and [2]Biotechnology, Parke-Davis Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

There are a variety of subtypes of voltage-gated Ca^{2+} channels that have been described using electrophysiological and molecular techniques. One subtype of Ca^{2+} channels, designated as N-type, is found predominantly on neurons and has been shown to be important in mediating neurotransmitter release as well as neuronal excitability. Expressed Ca^{2+} channels that contain α_{1B} (Class B) have pharmacological and biophysical properties that are similar to N-type Ca^{2+}channels. Voltage-dependent blockers of N-type Ca^{2+} channels may be useful in the treatment of neurological disorders, including stroke.

Microinjection of cRNAs encoding for human neuronal α_{1B}, human neuronal β and rabbit skeletal muscle α_2 subunits produced high voltage-activated Ca^{2+} channels in Xenopus oocytes, whose activity was measured using two electrode voltage-clamp techniques. The block of expressed channels by lidocaine and verapamil was done at holding potentials where there was a small degree (5%) of steady state inactivation (-80 mV) and at depolarized holding potentials (-55 mV) where approximately 50% of the channels were inactivated. Using these two holding potentials we found a 5-50 fold increase in the potency of these two compounds when inducing current from the more depolarized holding potential. The ω-conotoxin GVIA tested in the same paradigm blocked Class B Ca^{2+} channels, but showed no voltage-dependency. Lidocaine and verapamil are non-selective ion channel blockers, producing voltage-dependent blocks of sodium and L-type calcium (verapamil) channels.

These results suggest that lidocaine and verapamil interact with a common site on Na^+ and Ca^{2+} channels that is revealed under depolarization or inactivation.

799.6

ω-CONOTOXIN GVIA AND SPERMINE CAUSE THE SAME EFFECT ON HIPPOCAMPAL FIELD POTENTIALS, BUT SPERMINE DOES NOT PREVENT CONOTOXIN BINDING. P.A. Ferchmin*; E.M. Rivera and V.A. Eterović. *Center for Molecular and Behavioral Neuroscience, Department of Biochemistry, Univ. Central del Caribe, Bayamón, PR 00960.*

When stimulating in area CA1 of hippocampal slices with paired pulses, 1μM ω-conotoxin GVIA (ω-CTX) inhibited irreversibly the first, or conditioning population spike (PS) and increased paired-pulse facilitation. Similar but reversible effects were observed with 1-3 mM spermine, suggesting that it too inhibits N-type Ca^{++} channels. However, spermine did not protect PS from irreversible inhibition by ω-CTX, which suggests a binding site on the channel not overlapping with that of ω-CTX. Upon complete inhibition of PS by ω-CTX, increased stimulation restored a full PS but did not decrease the degree of paired-pulse facilitation. This restored PS was inhibited by spermine with an IC_{50} similar to that seen with naive slices. Since ω-CTX block is irreversible, strong stimulation could have recruited other types of Ca^{++} channels or induced a voltage-dependent relieve of ω-CTX inhibition; since spermine potency did not change, it may act as a general inhibitor of voltage-dependent Ca^{++} channels. Supported by NIH-MBRS GM50695 and NIH-RCMI RR03035.

799.7

INTERACTIONS BETWEEN VOLATILE ANESTHETICS AND VOLTAGE DEPENDENT CALCIUM CHANNELS IN ISOLATED RAT BRAIN SYNAPTOSOMES. F. Xu, J. Zhang, V. White, H.C. Hemmings, Jr.*, T.J.J. Blanck, Dept. of Anesthesiology, Cornell University Medical College, NY 10021.

Presynaptic depression of Ca^{2+} entry through CNS voltage dependent Ca^{2+}-channels (VDCC) leading to inhibition of neurotransmitter release may be a primary effect of volatile anesthetics (VA) during general anesthesia. This work describes a parallel inhibition of binding and Ca^{2+} influx caused by halothane and isoflurane and demonstrates interactions between VAs and inhibitors of L- and N-type VDCC. Halothane and isoflurane (0.25-1.5 mM) inhibited dose-dependently K^+-stimulated Ca^{2+} influx into rat cortical synaptosomes to 55% and 65% of control value, respectively. Both anesthetics also depressed [^{3}H]isradipine binding to synaptosome membranes dose-dependently over the same concentration range. In equilibrium binding studies, an increase of K_d and a decrease of B_{max} were observed in presence of 1.5 mM halothane or isoflurane compared to the control values. In the absence of volatile anesthetics, nitrendipine (NTP, an L-type Ca^{2+}-channel antagonist) caused a slight increase in K^+-stimulated Ca^{2+} influx into rat synaptosomes, whereas the N-type Ca^{2+}-channel blocker ω-conotoxin GVIA (CTX) inhibited Ca^{2+} influx dose-dependently. Simultaneous treatment with 1 μM NTP and 200 nM CTX resulted in an intermediate inhibition of Ca^{2+} influx. The inhibitory effect of halothane on Ca^{2+} influx was potentiated by NTP, by CTX, or, more strongly, by NTP and CTX simultaneously. In contrast, the inhibitory effect of isoflurane on Ca^{2+} influx was potentiated by simultaneous use of NTP and CTX, but not by NTP or CTX alone. These results suggest that the inhibitory effect of halothane and isoflurane on depolarization-evoked Ca^{2+} influx in rat cortical synaptosomes involves L- and/or N-type VDCC located on synaptosomal plasma membranes. Inhibition of VDCC may play an important role in the CNS effects of VAs.

799.8

MECHANISM OF ACTION OF CISSUS SICYOIDES WATERY EXTRACT ON MALE GUINEA PIG AORTIC RINGS. E. Gijón*, L. Cartas, M. Lorenzana-Jimenez, and X. García. Dept. of Physiology and Dept. of Pharmacology, School of Medicine. UNAM. México D.F. 04510. México.

The extract of Cissus sicyoides (Cs) from dry leaves 1:10 w/v produces vasoconstriction in aortic rings without endothelia in a dose dependent manner (1). As this could be explained by calcium movilization trough voltage dependent channels or receptor dependent channels, we tested several potassium chloride (KCl) concentrations. KCl 7.7-10 mM increased Cs contractil response while KCl 20-77 mM decreased this response. Cs vasoconstriction dose response curve may be observed in calcium free solutions with EGTA 0.2 mM and 3 mM and caffeine 5 and 50 mM. Caffeine blocked Cs contraction dose dependent. These suggests that Cs contraction depends on calcium channels activated by membrane voltage changes and on internal calcium deposits.

1. Proc. West. Pharmacol. Soc. 36:7-12, 1993.

799.9

LAMBERT-EATON MYASTENIC SYNDROME IgGs INHIBIT NON-L, NON-N Ca^{2+}-CHANNELS IN RAT INSULINOMA RINm5F CELLS. C. Grassi*, V. Magnelli^, E. Sher°, G.B. Azzena and E. Carbone^ Institute of Human Physiology, UCSC , Rome, Italy. ^Dept. of Anatomy and Human Physiology, University of Turin, Italy. °Center of Citopharmacology, CNR, Milan, Italy

Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disease characterised by a widespread disorder of neurotransmitter release, which has been attributed to down-regulation of presynaptic Ca^{2+}-channels. We have recently shown that LEMS IgGs reduce Ca^{2+}-currents through N-type channels and this might account for impairment of the autonomic functions observed in this syndrome (Grassi et al., *Neurosci. Lett.* 181:50-56, 1994). However, acetilcholine release at level of the mammalian neuromuscular junction has been proposed to be due to activation of non-L non-N Ca^{2+}-channels. In the present study we investigated the action of LEMS IgGs on Ca^{2+}-currents in RINm5F cells, which show a high percentage (30-35%) of non-L non-N Ca^{2+}-channels (Magnelli et al., *Pflügers Arch.*, 1995 in press). High-voltage activated (HVA) Ca^{2+}-currents were recorded in 10mM Ba^{2+} from cells incubated (12 h) with IgGs (2 mg/ml) of either LEMS or control subjects. After ω-CTx-GVIA-treatment (3.2μM for 15 min) and in the presence of nifedipine (5 μM), current amplitude was 84±12pA (S.E.M., n=18) in LEMS IgG-treated cells and 160±20pA (n=19) in controls (47% depression, P< 0.01). Activation and inactivation kinetics were not modified by LEMS IgGs. The channel voltage-dependency in control- and LEMS-IgG treated cells did not exhibit significant difference, derived from the absence of shift in the current-voltage (IV) curve. Our data suggest that LEMS IgGs target non-L, non-N Ca^{2+}-channels as well as other HVA Ca^{2+} channels thus probably causing the impairment of acetilcholine release at the neuromuscular junction level observed in LEMS patients.

799.10

PROPOFOL INDUCES EXTRA CELLULAR ACIDIFICATION IN NEURONS.

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The site of action of the anaesthetic drug propofol (Diprivan®) is still unknown. We have previously shown that propofol induces changes in the cytoskeleton arrangement after an intracellular calcium rise (1). Our hypothesises is that anaesthetic drugs interact with the cell membrane which change the state of the ion channels and induce changes in the cytoskeleton.

We have used a microphysiometer (2) to measure the amount of proton excretion in primary culture of neurons after stimulation with propofol in the concentration of 0.3, 3 and 30 μg/ml respectively. We used medium or 10% Intralipid® as controls.

To further investigate how the propofol-induced rise in intracellular calcium occur we used the tyrosine kinase inhibitor herbimycin A, incubated for 30 min, and thereafter measured intracellular calcium in single cells as described previously (1).

The results shows dose dependent and reversible changes in the extracellular acidification. We believe this acidification is caused by acidic metabolites leaving the cell through open ion channels, probably calcium channels. The intracellular calcium rise was reduced when herbimycin A was added. The reduction was caused by reduced influx of calcium ions. This shows that tyrosine kinase has a roll in the intracellular pathways caused by propofol.

1. Jensen A.G., Lindroth M., Sjölander A., Eintrei C: Anesthesiology 81:1220 - 1229, 1994
2. McConnell H. M., Owicki J:C: et al: Science 257: 1906 - 1912, 1992

800.1

GENETIC DISRUPTION OF THE M1 MUSCARINIC RECEPTOR IN MICE S. E. Hamilton*, M. Qi, G. S. McKnight, N. M. Nathanson, R. L. Idzerda. Department of Pharmacology, University of Washington, Box 357280, Seattle WA 98195-7280

The m1 muscarinic receptor is found in high concentrations in the hippocampus where it is thought to be involved in memory and learning. It is also present in sympathetic ganglia where it regulates synaptic transmission through the ganglia from the central nervous system (CNS) to the heart and blood vessels. We are generating mouse strains deficient in the m1 receptor in order to determine its function in the nervous system. An allele of this gene was "knocked-out" in mouse embryonic stem (ES) cells by homologous recombination using a 7 kb fragment of the mouse m1 gene in which the sequence corresponding to the N-terminal 56 amino acids was replaced with the sequence for the positive selectable marker, neomycin phosphotransferase. In addition the gene encoding HSV thymidine kinase was incorporated at one end of our construct as a marker for negative selection. After introducing the construct into ES cells by electroporation, colonies were grown in the presence of G418 and gancyclovir and were screened using both PCR and Southern analyses. Two of our cell lines deficient in m1 yielded chimeric mice and the mutant m1 gene was transmitted through the germline. The resulting heterozygotes are being bred to obtain homozygotes deficient in both copies of the m1 gene. We will verify the loss of m1 protein expression using an m1 specific polyclonal antibody we have generated and will use these mice to elucidate the role of m1 receptors in the CNS.

800.2

PRESENCE OF M_4 AND M_1 MUSCARINIC RECEPTOR SUBTYPES IN RAT OLFACTORY BULB AND M_4 MEDIATES BIMODAL CONTROL OF CYCLIC AMP FORMATION. H. Ladinsky*, G.B. Schiavi, M. Gil Quintero, M. Turconi, M.C. Olianas[1] and P.L. Onali[1]. Research Department, Boehringer Ingelheim Italia, Milan, Italy 20139 and [1]Department of Neurosciences, University of Cagliari, Cagliari, Italy 09124.

The pharmacology of muscarinic receptor (mAChR) sites in the rat olfactory bulb (OB) was characterized by use of a series of displacing ligands. In binding experiments against [^{3}H]NMS, regression analysis of the shallow inhibition curves produced by M_1-selective antagonists pirenzepine, guanylpirenzepine, DAG 5600 and DAG 5198 showed that M_1 mAChRs make up 47.4±2% of total receptors, and those produced by M_4/M_2-discriminating compounds DAU 6385 and DAU 6387 indicated that M_4 receptors comprise 45.2% of all mAChRs. The M_1+M_4 mAChRs thus make up 93% of the total population in OB. None of the affinity constants of the drugs indicated the presence of M_2 binding sites. Both carbachol-stimulated, and carbachol-inhibited Ca^{2+}/calmodulin- and forskolin-stimulated adenylate cyclase activity in OB homogenates were blocked by the above antagonists as well as by AF-DX 116, himbacine and AQ-RA 741. The inhibition constants of the antagonists, determined by Schild analysis, correlated highly ($R = 0.95$) with the binding affinities of the antagonists for the m4 mAChR in NG 108-15 neuroblastoma-glioma hybrid cells but not with those for the rat cortical M_1 ($R = 0.31$) or cardiac M_2 ($R = 0.76$) mAChRs. The results thus provide evidence that the M_4 subtype mediates the cholinergic bimodal regulation of cyclic AMP formation in OB.

800.3

m5 MUSCARINIC RECEPTORS ON MICROGLIA: IMPLICATIONS FOR CHOLINERGIC NEURON-MICROGLIAL COMMUNICATION. G. Ferrari-DiLeo and D.D. Flynn*. Dept. of Pharmacology, Univ. of Miami School of Medicine, Miami, FL 33101

Microglia, the resident macrophages of the CNS, are involved in both protective and destructive processes important for neuronal survival. The close association of microglia with basal forebrain cholinergic neurons and the presence of cholinergic muscarinic receptors on microglia suggest possible unique cholinergic neuron-microglia interactions. While there is evidence to suggest that microglial activation may be, in part, controlled by neurotransmitters and neuropeptides, little is known about the signal transduction mechanisms involved. We have previously demonstrated that the m5 subtype is the predominant muscarinic receptor on microglia isolated from adult and newborn brains and on blood macrophages (Ferrari-DiLeo & Flynn, *Life Sci.* 56:1037, 1995). In order to elucidate cholinergic regulation of microglia function, we have characterized the coupling of muscarinic receptor activation to signal transduction mechanisms in microglia. These studies are the first to examine m5 receptor signalling properties in a preparation that endogenously expresses this subtype. Carbachol stimulation of microglia results in the activation of PLC and release of inositol phosphates but not in the activation of PLA_2 or release of arachidonic acid. The efficiency of coupling of muscarinic receptor stimulation to nitric oxide synthase activation is being investigated. In order to examine putative microglia-to-neuron signalling, we determined the effects of microglia secreted factors on muscarinic receptor binding and second messenger responses. Cells exposed to microglia-conditioned media demonstrated decreased responses to carbachol. These studies suggest that not only do microglia respond to cholinergic stimulation but that microglia may modulate cholinergic neurotransmission.

800.4

SODIUM NITROPRUSSIDE INDUCES INTERNALIZATION OF MUSCARINIC RECEPTORS R. Maggio*, P. Barbier, F. Fornai, F. Vaglini and G. U. Corsini. Institute of Pharmacology, School of Medicine, University of Pisa, Italy.

In the present study we have extensively characterized the internalization of muscarinic acethylcholine receptors induced by the nitrogen monoxide (NO) generating compound sodium nitroprusside (SNP). When CHO cells stably transfected with the m4 muscarinic receptor subtype were incubated for 1 h in the presence of 700 μM SNP, the number of receptors, measured in intact cells with the hydrophilic ligand [^{3}H]N-methylscopolamine ([^{3}H]NMS), was reduced by 30 %. This effect was dose dependent, beginning with concentration of SNP as low as 45 μM. Receptor diminution induced by SNP was very fast ($t_{1/2}$ ranged between 10 and 20 min). Removal of SNP from the incubation medium did not result in a recovery of the binding sites measured with [^{3}H]NMS. The phenomenon was temperature dependent (it did not occur at 4 °C), and was blocked by the muscarinic antagonist atropine. Moreover, the effect of SNP was not observed in cell homogenate indicating that the integrity of the cell was required. Receptor diminution was not detected when the number of binding sites was evaluated with the lipophilic antagonist [^{3}H]quinuclidynil benzilate. This indicates that SNP induces a redistribution of the muscarinic receptors between the plasma membrane and an internal compartment of the cell. Receptor loss was readily reversed by the treatment with the sulphydryl reducing agent diethyldithiocarbamate (10 μM). Our data indicate that muscarinic receptors are internalized by SNP through the oxidation of sulphydryl groups; moreover they suggest that NO could play a role in muscarinic receptor desensitization.

800.5

CONSTITUTIVELY ACTIVE MUSCARINIC RECEPTORS PRODUCED BY RANDOM MUTAGENESIS OF THE SIXTH TRANSMEMBRANE DOMAIN. T. A. Spalding*, E. S. Burstein, D. Hill-Eubanks, M. R. Brann. Department of Psychiatry, University of Vermont, Burlington VT 05405 and Receptor Technologies, Winooski, VT 05404.

Libraries of recombinant m5 muscarinic receptors were constructed containing random mutations in regions of the receptor thought to be involved in ligand binding and G-protein coupling. Functional receptors were identified based on their ability to amplify NIH-3T3 cells in a ligand-dependant manner. We have isolated constitutively activated receptors which are able to amplify NIH-3T3 cells in the absence of ligand. This constitutive activity is inhibited by atropine and other classical muscarinic antagonists. The receptors remain sensitive to carbachol, which is ten to fifty times more potent on constitutively activated receptors compared to wildtype.

We have constructed libraries of receptors containing mutations in the third, fifth and sixth transmembrane regions, the N and C terminii of the third internal loop and the second internal loop of the receptor. However, all of the constitutively activated receptors identified to date contain multiple mutations in the sixth transmembrane domain. We are currently performing site directed mutagenesis studies to determine whether individual amino acid substitutions can cause the constitutive activation of the receptor.

800.6

NOVEL HIGH THROUGHPUT ASSAYS OF CLONED RECEPTOR PHARMACOLOGY IN LIVING MAMMALIAN CELLS. Terri L. Messier, Christine M. Dorman, Hans Braüner-Osborne, David Eubanks, S.V. Penelope Jones*, and Mark R. Brann. Receptor Technologies Inc. 276 East Allen Street Winooski, VT 05404.

An innovative high throughput assay of cloned receptor pharmacology has been developed based on the observation that many receptors stimulate proliferation of NIH 3T3 cells in a ligand dependent manner. We report application of this assay to evaluate the pharmacology of ligands for cloned receptors belonging to a wide range of functional and pharmacological classes. These include muscarinic, adrenergic, neurokinin, prostanoid, endothelian and neurotrophin receptors. Our assay involves the co-expression of an enzyme marker with the receptor of interest. The cloned receptor ligand stimulates proliferation of the cells and the induced effect is assessed by measurement of the co-expressed β-galactosidase. This assay is called Receptor Selection and Amplification Technology (R-SAT, patent pending). R-SAT offers significant advantages compared to other methods for evaluating receptor pharmacology. A colormetric change is measured in a 96-well plate format providing the ability to easily screen many ligands for activity toward one or more receptors in a single assay. A positive correlation is shown when we compare ligand activity in our assay and more traditional assays. In addition, our assay can measure the response of ligands toward a receptor in a relatively short period (4-6 days). This assay strategy facilitates screening of large compound libraries and can reliably discriminate between agonist, partial agonist, and antagonist. R-SAT, a novel high throughput assay provides a simple, rapid, and economical method of reliably screening compounds and receptors.

800.7

Isolation and Characterization of the chick m2 and mouse m1 muscarinic acetylcholine receptor promoters.

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We are interested in the molecular mechanisms and signal transduction pathways involved with the transcriptional regulation of muscarinic acetycholine receptors (mAChR's). We have isolated genomic regions containing the putative chick m2 (cm2) promoter and the putative mouse m1 (mm1) promoter.

Constructs containing the putative promoters drive the expression of the firefly luciferase gene when transiently transfected into IMR-32 human neuroblastoma cells. Treatment of cells for 8 hrs. with leukemia inhibitory factor (LIF) or ciliary neurotrophic factor (CNTF) results in an increase in cm2 driven luciferase expression while mm1 driven luciferase expression remains unchanged. Treatment with nerve growth factor (NGF) had no effect on expression of either reporter constructs. We have begun to compare the regulation of the endogenous mAChR levels and their respective mRNAs with the regulation of the reporter gene constructs.

Initial studies indicate the putative cm2 promoter will drive luciferase expression in transiently transfected chick heart primary cultures (>8 fold above vector alone). We are currently investigating the regulation of the cm2 luciferase constructs in this system.

800.8

DISTINGUISHING BETWEEN STERIC HINDRANCE AND ORDINARY COOPERATIVE RECEPTOR MODELS.

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Radioligand binding studies are often carried out to distinguish among alternative kinetic models of receptor binding. When there is knowledge of the 3-dimensional molecular structure of the receptor it may be possible to express the structural features in terms of kinetic rate constants. This specialized model may then be compared to a more general model to obtain statistical verification or rejection of the adequacy of the specialized, structurally-based model. As an example we compare between an ordinary cooperative receptor model and a cooperative receptor model with steric hindrance as recently proposed by Proska and Tucek (Mol. Pharmacol. 45: 709-717, 1994).

The starting parameters used for the models were obtained from real kinetic experiments of radiolabeled N-methyl-scopolamine at M2-muscarinic receptors of the rat heart as published by Proska and Tucek. Nonequilibrium pseudodata with random error (4%) were generated. Parameters were estimated by minimizing a weighted sum of squared errors with simultaneous analysis of pooled kinetic pseudodata. Statistical significance between the two models was calculated using the partial F-test. Successful statistical discrimination between the two models was obtained with a design consisting of association experiments performed with several different radioligand and several different inhibitor concentrations. In contrast, simulated pooled equilibrium experiments or pooled association experiments using only 1 radioligand concentration even with several inhibitor concentrations failed to distinguish between the two receptor models.

800.9

IDENTIFICATION OF SINGLE AMINO ACID RESIDUES DETERMINING SELECTIVE ACTIVATION OF $G_{q/11}$ BY THE m3 MUSCARINIC ACETYLCHOLINE RECEPTOR.

N. Blin, J. Yun and J. Wess*. National Institutes of Health, NIDDK, Lab. of Bioorganic Chemistry, Bethesda, MD 20892.

A large body of evidence suggests that the specificity of receptor/G protein interactions is determined by multiple intracellular receptor domains. To gain deeper insight into the molecular mechanisms governing receptor/G protein coupling selectivity, specific amino acids which are of particular importance for proper G protein recognition need to be identified. Towards this goal, the present study was undertaken to identify single amino acids required for selectively coupling the m3 muscarinic acetylcholine receptor to G proteins of the $G_{q/11}$ family. Distinct intracellular segments/amino acids of the m3 receptor were systematically substituted into the structurally closely related m2 muscarinic receptor which does not couple to $G_{q/11}$ but to $G_{i/o}$ proteins. The resultant mutant receptors were expressed in COS-7 cells and studied for their ability to induce agonist-dependent stimulation of phosphatidyl inositol hydrolysis, a response known to be mediated by G proteins of the $G_{q/11}$ class. Using this approach, we identified four amino acids in the second intracellular loop and four amino acids at the C-terminus of the third intracellular loop of the m3 muscarinic receptor which are essential for efficient $G_{q/11}$ activation. We could demonstrate that these amino acids, together with a short segment at the N-terminus of the third intracellular loop, fully account for the G protein coupling preference of the m3 muscarinic receptor. Taken together, our data strongly suggest that only a rather limited number of amino acids, located on different intracellular regions, are required to determine the functional profile of a given G protein-coupled receptor.

800.10

CHARACTERIZATION OF A CHICK m5 MUSCARINIC ACETYLCHOLINE RECEPTOR

S. Creason, K. Tietje, and N.M. Nathanson*. Department of Pharmacology, University of Washington, Seattle, WA. 98195

A partial length cDNA clone coding for a muscarinic acetylcholine receptor was isolated from chick heart using PCR and degenerate oligonucleotide primers. Sequence analysis showed this fragment to have 91% similarity and 88% identity with the third cytoplasmic loop of the rat m5 receptor. This fragment was used to probe a chicken genomic library to obtain a full length clone encoding the chick m5 (cm5) muscarinic receptor. The cm5 receptor, like all other vertebrate muscarinic receptors cloned to date, did not contain any introns in the coding region of the gene. cm5 specific oligonucleotides were used in reverse transcription PCR assays to demonstrate that the cm5 receptor is expressed in both chick heart and chick brain.

When expressed in stably transfected CHO cells, the cm5 receptor exhibited high affinity for the muscarinic receptor specific antagonist 3H-QNB. Ligand binding assays with perinzipine, AF-DX 116, and carbachol show this receptor to have pharmacological properties similiar to other cloned muscarinic receptors. Functional studies demonstrate the ability of this receptor to stimulate phospholipase C and increase carbachol stimulated phosphatidylinositol hydrolysis in stably transfected CHO cells.

800.11

SITE-DIRECTED MUTAGENESIS OF THE m1 MUSCARINIC RECEPTOR: ROLE OF B-B-X-X-B MOTIF IN RECEPTOR-G-PROTEIN COUPLING.

N.S.M. Geoghagen, R.T. Cline, C.M. Fraser and N.H. Lee*. Department of Molecular and Cellular Biology. The Institute for Genomic Research, Gaithersburg, MD 20878.

Seven transmembrane domain (7tmd) receptors modulate the activity of their effector enzymes via G-proteins. Examples of single tmd receptors that functionally interact with G-proteins are also known. Of interest is the finding that the cytoplasmic regions of these two structurally diverse class of receptors contain a B-B-X-X-B motif (where B is a basic amino acid and X is a non-basic amino acid) which is postulated to be the G-protein activator domain. Agonist-stimulation of m1 muscarinic acetylcholine receptors (m1mAChRs), belonging to the 7tmd receptor family, results in hydrolysis of phosphoinositides (PI). The carboxyl-terminal, cytoplasmic domains of m1mAChRs contain two of these G-protein-activator motifs, $KRTPR^{141}$ and $KKAAR^{365}$ (where the number indicates position of the last amino acid) in the 2nd and 3rd intracellular loops, respectively. We have mutated the basic amino acids to alanine (A) to access their role in receptor-G-protein coupling. Antagonist binding and expression levels (1-2 pmol/mg protein) of mutant receptors where comparable to wild-type m1mAChRs. The $AAAAA^{365}$, but not the $AATPA^{141}$, mutant receptor was functionally uncoupled for agonist-stimulated PI hydrolysis. The $AKAAR^{365}$ and $KKAAA^{365}$ mutant receptors exhibited decreased potency and efficacy to stimulate PI hydrolysis compared to wild-type m1mAChRs, respectively. The $KAAAR^{365}$ and $KAAAA^{365}$ mutants exhibited both decreased potency and efficacy to stimulate PI hydrolysis. These data demonstrate that the $KKAAR^{365}$ motif is the G-protein activator domain with each basic amino acid contributing to agonist-stimulated PI hydrolysis.

800.12

STRUCTURE OF THE m4 MUSCARINIC RECEPTOR GENE AND ITS PROMOTER.

Avtar. Roopra, Ian.C. Wood, Christina.A. Harrington* and Noel J. Buckley, Wellcome Laboratory for Molecular Pharmacology, University College London, London WC1E 6BT, U.K.

G-protein coupled receptors are encoded by one of the most diverse gene families in the mammalian genome, accounting for as much as 1% of the entire genome. Most, if not all, of these gene products have a unique distribution within the nervous system and hence it is of interest to determine how these expression profiles are brought about i.e. What determines the receptor repertoire of individual neurones?

Cholinergic muscarinic receptor genes are members of this gene superfamily. The m4 gene is expressed mainly in telencephalic regions of the CNS, autonomic ganglia and lung; Activation of m4 receptors can lead to closing of N-type voltage sensitive Ca^{2+} channels, activation of K^+ channels and inhibition of adenylyl cyclase. Analysis of the m4 gene has shown that it contains a 460bp non-coding exon separated from the single coding exon by a 4.8kb intron. A rat cosmid clone containing the m4 gene and 20kb of upstream sequence was found to be sufficient to direct expression of the m4 gene in a cell type specific manner when transfected into cell lines. A DNA sequence analysis of the 5'flanking region of the m4 promoter showed that this region is *gc* rich, does not contain a TATA or CAAT box and has several consensus sequences for enhancer elements including AP-2, AP-3 and E-Box binding sites. Transient transfection of a series of luciferase reporter constructs into different cell lines demonstrated that 600bp of 5'flank was capable of driving cell specific expression. Present studies are aimed at defining those *cis* elements which are responsible for driving cell specific expression of the m4 gene and functionally assessing their role both *in vitro* and in transgenic mice.

800.13

m1-m4 MUSCARINIC RECEPTOR PROTEINS IN RAT DENTATE GYRUS AND AFFERENT PROJECTION NEURONS. S.T. Rouse* and A.I. Levey. Dept. of Neurology, Emory Univ. Sch. of Med., Atlanta, GA 30322

Acetylcholine (ACh) has a variety of modulatory roles in the hippocampus, which are mediated by a family of five mAChR subtypes (m1-m5). To establish the anatomical basis of ACh actions, we studied the expression of mAChRs in the dentate gyrus (DG) using light microscopic immunocytochemistry (ICC) (Levey, 1991). m1-m4 subtypes are found in the neuropil of the molecular layer, but with differential laminar distributions. m1 and m3 are expressed throughout the molecular layer and in granule cell bodies, suggesting, at least in part, the postsynaptic distributions of these subtypes. m2 is concentrated in a very thin band at the junction of the inner and middle third of the molecular layer. m4 is enriched in the inner third of the molecular layer, suggesting localization in associational/commissural projections. All 4 subtypes were identified in hilar neurons.

To identify the receptor proteins in hippocampal projection neurons, we injected retrograde tracer (WGA-HRP) unilaterally into the DG, combined with mAChR ICC. Multipolar hilar cells of the contralateral hippocampus that comprise the commissural pathway express m1, m3 and m4, but not m2. Septohippocampal neurons express the m2 subtype. Raphe cells that project to the DG express the m1 subtype. Cells in layer 2 of the entorhinal cortex, that comprise the perforant pathway, express m1, m2 and m4. These results indicate mAChR subtypes are differentially expressed in DG and its afferent projection neurons. Future studies will delineate the pre- and postsynaptic distributions of the subtypes in these circuits.

800.14

MUSCARINIC CHOLINERGIC RECEPTOR (Hm1) INTERNALIZES VIA CLATHRIN-COATED VESICLES. L.M. Tolbert, R.I. Cone* and J. Lameh, Departments of Pharmacy and Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446.

Upon exposure to agonists such as carbachol, muscarinic receptors quickly internalize to a significant degree. The mechanism of muscarinic receptor internalization is poorly understood. We have used immunofluorescence confocal microscopy to identify the pathway by which muscarinic receptors are internalized. Using human embryonic kidney cells (HEK 293) transfected with the Hm1 (human muscarinic subtype 1) receptor tagged with the epitope EYMPME, we have seen that acid treatment, which has been shown to inhibit internalization via clathrin-coated vesicles, inhibits carbachol-stimulated internalization. PMA, on the other hand, which inhibits caveolae-mediated endocytosis, has no effect on carbachol-induced endocytosis. Double-labeling studies with clathrin; α-adaptin, which links endocytosed proteins to clathrin; and transferrin, which recycles through clathrin-coated vesicles, show that each of these proteins is colocalized with Hm1 following agonist treatment. Conversely, in double-labeling studies with caveolin, the protein that comprises caveolae, no colocalization between Hm1 and caveolin was observed. These results suggest that agonist-induced internalization of the Hm1 receptor occurs by a clathrin-mediated pathway. Supported by MH 00996. L.M.T. supported by AFPE and GM08338.

800.15

EFFECT OF CARBACHOL ON MORPHOLOGICALLY CHARACTERIZED AMYGDALOID NEURONS: ANALYSIS "IN VITRO". J. Yajeya*[1], A. de la Fuente Juan[1], M. Merchán[2], I. Plaza[2], M. Heredia[1], A.S. Riolobos and J.M. Criado. Depts de Fisiologia y Farmacologia[1] and Biología Celular y Patología[2]. Universidad de Salamanca. 37007 Salamanca. Spain.

The cholinergic system of neurotransmission has been implicated in processes of attention, learning and memory. This system is connected with the amygdala, a structure also implicated in such processes. Using the slice technique, we have made intracellular recordings and tracer injections to determine: 1) the effects and action mechanisms of carbachol on basolateral neurons, 2) the morphological features of the neuron types affected. In 85 % of the one hundred and twenty-three neurons studied, carbachol (20 μM) induced a slow depolarization of 5-10 mV. Sometimes the cell attained the firing threshold This response was maintained under perfusion with Ba^{2+} (1mM) and using cesium acetate (3M) filled electrodes. The addition of flunarizine (10 μM) or the perfusion with low Ca^{2+} (0.2 mM) and high Mg^{2+} (2.4 mM) solutions did not block the depolarizing effect of carbachol, but changed the time course. The response was blocked under perfusión with T.E.A. (5 mM); it was also reduced in amplitude with T.T.X. (1 μM) perfusion or in solutions with a low Na^+ (58.5 mM). Biocytin-injected, carbachol affected neurons are multipolar, with spiny dendrites and pyramidal appearance, possibly corresponding to type I of McDonald. These results suggest that carbachol effects on basolateral amygdaloid neuron are mediated by modification of Na^+ and K^+ conductances. The Ca^{2+} ion may be involved in the maintenance of the long time course of the response under physiological conditions.

800.16

THREE TYPES OF ATROPINE-SENSITIVE RESPONSES TO CARBACHOL IN ACUTELY ISOLATED SEPTAL NEURONS OF THE RAT. L. R. Sun and J. B. Suszkiw*. Dept. of Molecular and Cellular Physiology, Univ.of Cincinnati College of Medicine, Cincinnati, OH 45267-0576.

Using whole cell current-clamping and voltage-clamping techniques, we investigated the responses of septal/diagonal band neurons to carbachol. Recordings were made from large (diameter ≥ 25 μm), acutely dissociated neurons from 14-22 day old Sprague-Dawley rat pups. Carbachol was applied (100 μM, 100 ms) to the cell via a puffer pipette before and following bath superfusion with 10 μM atropine. Responses to glutamate (100 μM, 100 ms) were measured as well. Glutamate elicited depolarizing, inward currents in all cells tested (50/50). Carbachol induced hyperpolarizing, outward currents in 27/50 (54%) of cells and depolarizing, inward currents in 6/50 (12%) of cells. In 5/50 (10%) of cells, carbachol increased membrane resistance that was accompanied by a increased firing frequency of action potential. All the responses were attenuated in the presence of atropine. Twenty four percent of the cells (12/50) didn't show any responses to carbachol. These results indicate that majority of large neurons in the septum are cholinoceptive and that three types of atropine-sensitive responses can be distinguished.
(Supported by NIEHS grant ES06365)

800.17

A VOLTAGE AND CALCIUM-DEPENDENT CATION CURRENT UNDERLIES THE DEPOLARIZATION AND DEPOLARIZING AFTER-POTENTIAL ELICITED BY MUSCARINIC ACTIVATION IN RAT CORTEX. S. Haj-Dahmane* and R. Andrade. Dept. of Pharmacol. and Physiol. Science, St. Louis Univ. School of Med. St. Louis, MO 63104.

Muscarinic stimulation depolarizes pyramidal neurons of the rat prefrontal cortex and induces the appearance of a slow calcium-activated afterdepolarization (sADP). We have previously shown that both of these effects reflect the activation of cation-nonselective currents. We now have examined the relationship of the currents underlying these two responses.

Whole cell recordings were obtained from layer V pyramidal neurons of rat prefrontal cortex in brain slices. Administration of carbachol elicited an inward current that increased steeply in amplitude above rest (approx -70 mV). Blocking calcium influx using cadmium greatly reduced this voltage dependence. Buffering of the intracellular calcium with 10 mM EGTA or BAPTA had no effect on the carbachol induced current (I_{CARB}) at rest. However it strongly reduced the amplitude of this current at depolarized potentials as well as the current underlying the after-depolarization (I_{ADP}). This indicated that the voltage dependence of I_{CARB} was due, at least partly, to calcium influx. Moreover it suggested the sADP resulted from a calcium induced enhancement of the current responsible for the depolarization. Consistent with this possibility, administration of TEA(2-3 mM), which blocks of depolarization, also inhibited the sADP.

These results suggest that muscarinic receptors activate a voltage and calcium-dependent cation current to depolarize cortical pyramidal cells. Calcium influx during one or more action potentials enhances this current and results in the appearance of a depolarizing afterpotential. Supported by MH 49355.

800.18

MUSCARINIC RECEPTORS MEDIATING INWARD AND OUTWARD CURRENTS IN RAT DORSOLATERAL SEPTAL NEURONS. T. Akasu*[1], H. Hasuo[1] and J. P. Gallagher[2] [1]Dept. Physiol., Kurume Univ. Sch. Med., Kurume 830, Japan and [2]Dept. Pharmacol. & Toxicol., Univ. Texas Med. Br., Galveston, Texas 77555, U.S.A.

Cellular mechanism and receptor type responsible for the muscarine-induced currents in dorsolateral septal nucleus (DLSN) neurons were investigated by using slice patch-clamp technique. Bath-application of muscarine (3-100 μM) caused either inward current (I_{mi}) or outward current (I_{mo}) in DLSN neurons. These currents were associated with increase in membrane conductance and were voltage-independent. The reversal potentials of I_{mi} and I_{mo} were -17.0±5.3 mV (n=14) and -90±4.3 mV (n=28), respectively. In subpopulation of neurons, muscarine caused the inward current by suppression of a voltage-dependent, non-inactivating K^+ current, the M-current. The I_{mi} was concentration-dependent; the EC_{50} of I_{mi} and I_{mo} were 23 and 7 μM, respectively. Atropine (0.2 μM) completely reduced both I_{mi} and I_{mo}. Pirenzepine (PZP) also reduced the I_{mi} and the I_{mo} in a competitive manner. Schild plot showed that the Kd for PZP was 120 nM (n=5) in the I_{mi}. Methoctramine (1 μM) competitively depressed the I_{mi} with Kd of 230 nM. AF-DX 116 (1 μM) produced no significant inhibition of the I_{mi}. In contrast, the Kd value for PZP was 54 nA in I_{mo}. AF-DX 116 inhibited the I_{mo} in a competitive manner with Kd of 410 nM. McN-A-343 produced neither inward nor outward current. Intracellular dialysis with GTPγS, a non-hydrolyzable analogue of GTP, irreversibly depressed both the I_{mi} and the I_{mo}. Pre-treatment of DLSN neurons with pertussis toxin (PTX) did not prevent the I_{mi} (n=8), while it completely depressed the I_{mo} (n=8). These data suggest that muscarine causes non-selective cation and K^+ currents acting at M_3 and M_4 subtype receptors, respectively, through different types of G-proteins in DLSN neurons.

800.19

A QUINUCLIDINE DERIVATIVE INDUCES AN INWARD CURRENT ASSOCIATED WITH M-POTASSIUM CURRENT INHIBITION IN m1-MUSCARINIC RECEPTOR-TRANSFORMED NG108-15 CELLS.

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3-(m-phenoxybenzylidene)-quinuclidine (KST-5452) is an M_1-specific ligand with an affinity constant of about 23 nM for rat cerebral cortical membrane. Oral administration of KST-5452 to rats with basal forebrain lesions improves passive avoidance performance. This suggests that KST-5452 has an ameliorating action on amnesia, probably through an effect on the cholinergic central nervous system. The electrophysiological effects of KST-5452 were studied in NG108-15 neuroblastoma x glioma hybrid cells transfected with m1 muscarinic acetylcholine receptor (mAChR) cDNA. Application of 0.1-1 mM KST-5452 (3 µl) on NGPM1-27 cells induced a long-lasting inward current accompanied by a fall in membrane conductance. The average amplitude of the inward current elicited by 1 mM KST-5452 was 0.6 ± 0.3 nA (n=19). Outward current was not observed in 19 NGPM1-27 cells tested. The inward current was accompanied by an inhibition of the amplitude of M-current relaxations (49 ± 11 %, n=10). The results show that KST-5452 mimics only an excitatory part of the ACh-inducd responses in m1-transformed cells and suggest that the ameliorating effect of KST-5452 on amnesia may be due to the increased membrane excitation in brain neurones which results from the inhibition of the M-current.

800.20

MUSCARINIC ENHANCEMENT OF A NOVEL NEURONAL CHLORIDE CONDUCTANCE.

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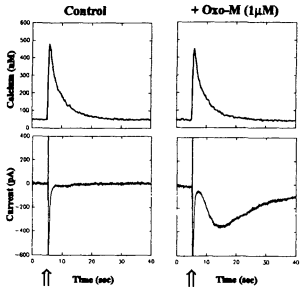
Experiments on cultured mamalian sympathetic neurons, using combined techniques of voltage-clamp perforated-patch recording and microfluorimetric measurements of intracellular Ca^{2+} with indo-1, have disclosed a delayed Cl-current that is synergistically regulated by the actions of raised intracellular Ca^{2+} and muscarinic (m1) receptor-mediated activation of protein kinase C (PKC).



In voltage-clamped (-60mV) Cs-loaded cells at 32 °C, bath application of the muscarinic receptor agonist oxotremorine-M (Oxo-M) did not change the resting intracellular Ca^{2+} concentration or membrane conductance but augmented a slowly developing inward current following an intracellular Ca^{2+} transient induced by a voltage-step (0.2s to 0mV, ⇑ in figure). This effect was mimicked by phorbol esters and blocked by down regulation of PKC and the PKC inhibitor calphostin C. Chloride channel blockers and ion substitution experiments suggest that the slow inward current results from an increased Cl^- conductance.

We also found that a brief application of acetylcholine could induce the delayed chloride current through a conjoint action on both nicotinic receptors (to produce a change in $[Ca^{2+}]_i$) and muscarinic receptors (to increase DAG levels and thereby activate PKC). This demonstrates an unusual form of synergism between two effects of a single transmitter

ACETYLCHOLINE RECEPTOR MUSCARINIC: AGONIST/ANTAGONIST FOR RECEPTORS

801.1

MUSCARINIC RECEPTOR INHIBITION OF AGONIST-STIMULATED CYCLIC AMP IN GUINEA PIG ILEUM.

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The longitudinal muscle of the guinea pig ileum contains both M_2 and M_3 muscarinic receptor subtypes. The M_3 subtype couples to phosphoinositide hydrolysis and elicits a direct contraction, whereas the more abundant M_2 subtype inhibits adenylate cyclase and has an indirect role in contraction. In rat ileum only forskolin, isoproterenol, PGE_1 and PGE_2 increased cAMP accumulation, and of these, only responses elicited by forskolin and isoproterenol could be opposed by the M_2 receptor (Griffin *et al., J. Pharmacol. Exp. Ther.* 263(1): 221-5, 1992). The purpose of this study was to characterize similar relationships in the guinea pig ileum. We investigated the ability of oxotremorine-M (oxo-M) to inhibit cAMP accumulation in slices of the guinea pig ileum in the presence of various agonists known to stimulate adenylate cyclase. Appreciable stimulation of cAMP (>50% over basal) was observed with maximal concentrations (1-10 µM) of forskolin, isoproterenol, PGE_1, PGE_2 and PGI_2, with fold stimulations over basal of 14.93, 2.51, 2.27, 2.28 and 1.52, respectively. Moderate cAMP stimulation (25 - 50% over basal) was seen using dopamine, 5-HT, 5-methoxytryptamine, dimaprit and VIP. Little or no effect (<25% over basal) was observed with SKF-38393, 2-chloroadenosine, CGS-21680, PGD_2, secretin and vasopressin. Oxo-M (1 µM) inhibited cAMP accumulation by 35% under basal conditions, while forskolin- and isoproterenol-stimulated cAMP was inhibited by 73 and 61%, respectively. Oxo-M inhibited PGE_1- and PGE_2-stimulated cAMP by 48 and 56%, respectively, but only inhibited PGI_2-stimulated cAMP by 33%, a value no greater than the oxo-M effect on basal cAMP. Oxo-M inhibition of cAMP accumulation in the presence of the other agonists with moderate or no stimulation of adenylate cyclase was less than or equal in magnitude to its effects on basal cAMP. Our results show that only forskolin, isoproterenol, PGE_1, and PGE_2 cause an appreciable increase in cAMP that is opposed by muscarinic stimulation, suggesting that the relaxing effects of these agents may be specifically inhibited by the M_2 receptor. (Supported by NIH grant NS30882)

801.2

Sequestration of muscarinic acetylcholine receptors.

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Sequestration of muscarinic acetylcholine receptors (m1-m5), which was assessed as loss of [^{3}H]N-methylscopolamine binding activity from the cell surface, was examined in COS-7 cells that had been transiently transfected with muscarinic receptors and G protein-coupled receptor kinase (GRK2 = βARK1) or GRK2 dominant-negative mutant (DN-GRK2). The agonist-dependent phosphorylation and sequestration of m2 receptors were facilitated by co-expression of GRK2, and reduced by co-expression of DN-GRK2. The sequestration of m4 receptors was observed by treatment with 10^{-5} M or higher concentrations of carbamylcholine and 40% of m4 receptors were sequestered by treatment with 10^{-4}-10^{-3} M of carbamylcholine for 2 hours. When GRK2 was co-expressed, 35% of m4 receptors were sequestered by treatment with 10^{-6} M of carbamylcholine and 65% of m4 receptors were sequestered by treatment of 10^{-4} -10^{-3} M of carbamylcholine for 2 hours. Co-expression of DN-GRK2 reduced the sequestration of m4 receptors to less than 25%. For m1, 3, 5 receptors, only less than 25% of receptors were sequestered by treatment of 10^{-4} -10^{-3} M of carbamylcholine for 2 hours. These results indicate that the phosphorylation of m2 and m4 receptors by GRK2 facilitates their sequestration and the mechanism of sequestration may be different between m2, 4 receptors and m1, 3, 5 receptors.

801.3

BUTYLTHIO[2.2.2]: AN ORALLY ACTING MUSCARINIC ANTINOCICEPTIVE IN RODENTS.

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Butylthio[2.2.2](+)-3-[4-(butylthio)-1,2,5-thiadiazol-3-yl]-1-azabicyclo [2.2.2]octane) is a muscarinic agonist/antagonist at muscarinic receptors in vitro. The analgesic effect of butylthio[2.2.2] was assessed in the Grid Shock, Tail Flick, Hot Plate, and Writhing tests in mice 30 min after s.c. or p.o. administration. ED_{50}'s (mg/kg) ranged from 0.12 - 3.5 (s.c.) and 1.15 - 19.0 (p.o.), yielding s.c./p.o. ratios of 5 - 20. ED_{50}'s (mg/kg) for the muscarinic side effects salivation or tremor were 13.5 (s.c.) and >60 (p.o.), yielding therapeutic windows > 50. Butylthio[2.2.2] was equieffective too, and 3 - 23 times more potent than morphine. Butylthio[2.2.2]'s duration of action was similar to morphine's (160 min). The dose response curve was shifted to the right by the muscarinic antagonist scopolamine but not by the opioid antagonist naltrexone. After 6.5 days repeated dosing there was marked tolerance to morphine, but less, if any, to butylthio[2.2.2]. In the rat Grid Shock ED_{50}'s (mg/kg) of 0.3 (s.c.) and 22.0 (p.o.) mg/kg were obtained. These data show that butylthio[2.2.2] potently and efficaciously produces antinociception after s.c and p.o. administration in mice and rats.

801.4

MOLECULAR STUDIES OF AGONIST INTERACTIONS WITH m1 MUSCARINIC RECEPTORS.

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Selective muscarinic agonists might be useful in the treatment of Alzheimer's disease. Previous studies identified several amidine derivatives as efficacious m1 agonists. For example, 5-(3-methyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine (CDD-0098) displays high activity at m1 receptors. To explore the structure activity relationships of muscarinic receptor ligands, a novel isoxazole derivative was synthesized and tested for m1 receptor affinity and activity in A9 L cells.

5-(3-Methyl-isoxazol-5-yl)-1,4,5,6-tetrahydropyrimidine (CDD-0190) inhibited [^{3}H]-(R)-QNB binding to m1 receptors in a biphasic manner with 59 % of the sites at 1.6 µM and 41 % at 60 µM. CDD-0098 displayed higher affinity; 31 % at 21 nM and 69 % at 2.3 µM. CDD-0190 also stimulated PI metabolism through m1 receptors with an EC_{50} of 92 µM and maximal stimulation at 290 % above basal levels. CDD-0098 was both more potent (5.4 µM) and efficacious (640 %) in stimulating PI turnover in A9 L cells.

Molecular modeling studies, using a model of the m1 muscarinic receptor developed by Nordvall and Hacksell (*J. Med. Chem.* **36**:967-976, 1993), suggested that the isoxazole exhibits lower activity due to the loss of a hydrogen bonding interaction with threonine192 of the m1 receptor. The data provide the basis for a systematic evaluation of the molecular features important for binding and activity at muscarinic receptor subtypes.

This work was supported by NS 01493 and NS 31173.

801.5

FUNCTIONAL CHARACTERIZATION OF A SERIES OF NOVEL 1,4,5,6-TETRAHYDROPYRIMIDINE DERIVATIVES AS SELECTIVE MUSCARINIC AGONISTS. A.A. El-Assadi*, S.M. Abunada, R. Sehgal, K. Ryan, M.A. Shepherd and W.S. Messer, Jr. Department of Medicinal & Biological Chemistry, Center for Drug Design & Development, College of Pharmacy, The University of Toledo, Toledo, OH 43606

Selective muscarinic agonists might be useful in the treatment of Alzheimer's disease. Previous studies identified several amidine derivatives as selective and efficacious m1 agonists. 5-Ethyloxycarbonyl-1,4,5,6-tetrahydropyrimidine (CDD-078), 5-propargyloxycarbonyl-1,4,5,6-tetrahydropyrimidine (CDD-097), and 5-(3-ethyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine (CDD-102) display high efficacy at m1 receptors and lower activity at m3 receptors expressed in A9 L cells.

Functional selectivity for M_1 receptors was confirmed through a series of biochemical and behavioral studies. Pirenzepine was more effective than either AF-DX 116 or p-F-HHSiD in blocking the stimulation of PI turnover in rat cerebral cortex by each agonist. Pharmacological screening indicated few cholinergic side-effects (e.g., salivation, hypothermia) for each agonist following i.p. injections in mice. Finally, i.p. injections of CDD-097 (1.0 mg/kg) reversed spatial memory deficits induced by i.c.v. administration of hemicholinium-3 (5 µg) in a modified Morris water maze task. Taken together, the data indicate the functional selectivity of these compounds, which warrant further development as M_1 agonists for the treatment of Alzheimer's disease.

This work was supported by NS 01493 and NS 31173.

801.6

MUSCARINIC AGONIST STIMULATION OF CYCLIC AMP ACCUMULATION IN PERTUSSIS TOXIN-TREATED CHO-m4 CELLS. Kris Eckols and Neil DeLapp*, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

Of the five molecularly defined subtypes of muscarinic receptors, the m2 and m4 proteins are negatively coupled to adenylate cyclase. However, Jones et. al.(Mol Pharm 40: 242-247, 1991) reported a large atropine-inhibitable increase in cAMP formation induced by the muscarinic agonist carbachol in CHO-m4 cells that had been treated with pertussis toxin (PTX). The purpose of the present study was to characterize the pharmacology of muscarinic-mediated cAMP accumulation in PTX-treated CHO-m4 cells. Subconfluent CHO-m4 cells were treated for 18 hours with 100 ng/ml PTX. Cells were harvested with trypsin and were incubated in suspension for ten minutes in the presence of 1 µM forskolin ± the compounds tested. Incubations were terminated by boiling and cAMP was determined with an enzyme immunoassay. Antagonist dose-response curves were determined for reversal of stimulation by 10 µM oxotremorine M. Oxotremorine M stimulated an 8-10 fold increase in cAMP formation (EC50: 2.2 ± 1.1 µM) that was blocked by 1 µM atropine. A series of standard agonists exhibited a wide range of partial agonist activities (relative to oxotremorine M as 100): acetylcholine, 106; carbachol, 92; arecoline, 77; S-aceclidine, 69; muscarine, 63; bethanechol, 42; McN-A-343, 33; R-aceclidine, 13; pilocarpine, 11; and RS 86, 7. Estimated Ki's for antagonists were: atropine, 1.3; 4-DAMP, 3.6; dicyclomine, 40; PFHHSiD, 175; pirenzepine, 180; and AFDX 116, 1490. The rank order of antagonist potencies was the same as reported for m4-mediated inhibition of adenylate cyclase in rat striatum (Olianas and Onali, J. Pharm. Exp. Ther. 259: 680-686, 1991). Thus, in CHO-m4 cells lacking a functional Gi protein, the robust muscarinic stimulation of cAMP formation (16-20 fold over inhibition of forskolin-stimulated cAMP formation in non-PTX-treated cells) has a typical m4 pharmacology and readily discriminates full from partial muscarinic agonists.

801.7

NEOSTIGMINE APPEARS TO ACTIVATE MUSCARINIC RECEPTORS ON MUDPUPPY PARASYMPATHETIC POSTGANGLIONIC NEURONS. J.C. Hardwick, R.L. Parsons and S.B. Backman.* Dept. of Anatomy & Neurobiology, University of Vermont, Burlington, Vermont, 05405 and Dept. of Anaesthesia, Royal Victoria Hospital, McGill University, Montreal, Quebec, H3A 1A1.

Indirect evidence from *in-vivo* studies in cats suggests that the bradycardia produced by systemic administration of the anticholinesterase neostigmine may be mediated, in part, by direct activation of muscarinic receptors on cardiac parasympathetic postganglionic neurons (J Pharm Exp Ther 1993 265:194). In the present investigation, this possibility was examined more directly by studying the effect of direct application of neostigmine to individual parasympathetic postganglionic neurons of the mudpuppy (*Necturus maculosus*) cardiac ganglia. Previous studies in these neurons have shown that muscarinic agonists activate an inwardly rectifying potassium conductance via m2 receptors (Neuropharmacol 1992 31:1311). Neurons were impaled with an intracellular recording electrode and the voltage responses to brief (1-3 sec) pressure ejections of either neostigmine (10^{-4}M) or the muscarinic agonist oxotremorine (10^{-4}M) were examined. Neostigmine and oxotremorine applications produced hyperpolarizations of 20.4 ± 3.2 mV, n=10 & 30.2 ± 3.3 mV, n=8, respectively). Neither response was affected by superfusion with the nicotinic antagonist hexamethonium (100 µM). However, the neostigmine-and oxotremorine-induced hyperpolarizations were reversibly inhibited by both non-selective (atropine 1 µM) and selective m2 (gallamine 20 µM; AFDX-116 1 µM) muscarinic antagonists. The neostigmine-induced hyperpolarization was associated with a decrease in input conductance and appeared to reverse near the potassium equilibrium potential. These results suggest that neostigmine may activate m2 receptors on cardiac parasympathetic postganglionic neurons, independent of its anticholinesterase activity. Supported by NIH grant NS 23978 to R.L.P.

801.8

NOVEL MUSCARINIC M1 RECEPTOR AGONISTS PROMOTE SURVIVAL OF CNS NEURONS IN PRIMARY CELL CULTURE. J. Alberch*[1,2], D. Gurwitz[3,4], A. Fisher[3] and H.T.J. Mount[1]. [1]Dept. of Neuroscience and Cell Biology, Robert Wood Johnson Medical School-UMDNJ, Piscataway, NJ 08854, [2]Dept. de Biologia Cel.lular i Anatomia Patologica, Univ. Barcelona, Spain, [3]Israel Institute for Biological Research, Ness-Ziona, Israel and [4]Sackler Faculty of Medicine, Tel-Aviv University, Israel.

We reported previously that muscarinic receptor (mACh-R) activation increases survival of cultured Purkinje cells, grown in the presence of NGF (Mount *et al.* 1994, J. Neurochem. *63*, 2065). In the present study, we examined the trophic potential of a novel series of specific m1ACh-R partial agonists in primary cultures of cerebellum and striatum. These compounds (AF150(S), AF151(S) and AF102B) stimulate release of arachidonic acid and activate phospholipase C, but do not affect adenylyl cyclase in cell lines transfected with m1AChR gene.

Dissociated cultures of gestational day 18 (E18) rat cerebellum were grown for 6 days in the presence of drug +/- NGF. The m1AChR agonists elicited dose-dependent increases in Purkinje cell survival, that were pirenzepine (10 nM) sensitive. NGF potentiated the trophic action of low agonist concentrations (1-10 µM). NGF also improved Purkinje survival, when administered in combination with exogenous arachidonic acid.

To examine whether muscarinic mechanisms regulate survival in other brain areas, agonists were tested in cultures of E18 striatal neurons. AF150(S) elicited dose-dependent increases in cell number, at 7 days *in vitro*. Improvements in survival were paralleled by enhancement of [^{3}H]GABA uptake, suggesting that GABAergic neurons were affected.

In sum, m1ACh-R activation improves survival of diverse populations of cultured primary neurons. Receptor coupling to phospholipase C and/or arachidonic acid release may underlie these actions. (Supp: Medical Research Council [Canada] and Ministerio de Educacion y Ciencia [Spain].)

801.9

METHOXYPHENETHYLAMINE-ERGOLINE "HYBRIDS": NOVEL MUSCARINIC LIGANDS. M.A. Mayleben[1*], A. Monte[2], W. Roeske[3], H. Yamamura[3], R.B. Mailman[1], and D.E. Nichols[2]. Univ. of North Carolina[1], Chapel Hill, NC 27599, and Purdue Univ.[2], W. Lafayette, IN. 47907, Univ. of Arizona[3], Tempe, AZ

Among the deficits seen in Alzheimer's Disease are decreased levels of acetylcholine (ACh) in several brain regions. Attempts to remedy this deficit using either increased dietary choline or cholinesterase inhibitors have had very limited success. Direct pharmacotherapy has been plagued by the lack of selectivity of available drugs in discriminating among the five muscarinic receptors (m1-m5) that have been cloned to date. All of these receptors are in the G-protein superfamily, with the m1 and m3 receptors linked to PI hydrolysis, and the m2 and m4 receptors negatively coupled to adenylate cyclase (AC). Localization studies indicate that the muscarinic receptors are found in the heart (mostly m2), salivary and sweat glands, and brain. In the brain, m2 receptors are found predominantly presynaptically, while the m1 receptors are found postsynaptically. This pattern has suggested that an ideal Alzheimer's drug would be an agonist at the m1 receptor while having no affinity or even antagonistic effects at the m2 receptor. During the screening of aminotetrahydronapthofurans (NAPs) designed as serotonergic ligands, certain members were found to have nanomolar affinity at the m1 muscarinic receptor in brain, with seven times lower m2 and little m3 activity. These compounds were studied further in a murine fibroblast cell line (B82) transfected with either the m1 or the m2 receptor. In these lines, bromoNAP had higher affinity (m1 K_I = 14 nM; m2 K_I = 30 nM) than the parent drug (NAP) (m1 K_I = 149 nM; m2 Ki = 335 nM), whereas a second analog (N,N-dimethylNAP) had little affinity for these receptors (m1 K_I = 846 nM; m2 K_I = 943). Functional studies at both the m1 and m2 receptors demonstrated that all of these drugs were antagonists. Although these functional characteristics suggest the compounds will not have utility as therapy in AD, this class is unusual for high affinity muscarinic ligands in that they are primary amines.

801.10

IN VIVO EFFECTIVENESS OF SELECT M_2 ANTAGONISTS. M.J. Stillman*[1], B. Shukitt-Hale[1], A. Levy[2], and H.R. Lieberman. Military Performance and Neuroscience Division, United States Army Research Institute of Environmental Medicine, Natick, MA 01760-5007, [1]GEO-CENTERS, INC., Newton Centre, MA 02159, and [2]IIBR, Ness Ziona, ISRAEL.

The central cholinergic system has been shown to exhibit presynaptic modulation of acetylcholine (ACh) release. Although still under investigation, evidence from physiological and pharmacological studies suggests that the M_2 receptor subtype acts as a negative autoreceptor. This *in vivo* microdialysis study examined the effects of the M_2 antagonists, methoctramine, AF-DX 116, AF-DX 384, and AQ-RA 741, on hippocampal extracellular ACh levels. Drug (2, 4, 8, or 16 µM) or vehicle (Ringer's solution) was perfused via a microdialysis probe into the CA1 hippocampal region of freely-moving male Fischer 344 rats. Levels of ACh were assessed by HPLC-EC. All compounds significantly elevated ACh levels. When the dose-response functions were expressed as Kd multiples, the drugs were found to be on the same linear dose-response curve, with certain exceptions. These results support the concept that the proposed presynaptic mechanism of action of these drugs is M_2 receptor-based.

801.11

AN AGONISTIC EFFECT OF ATROPINE. P.S. Guth*, F.R. Domer, C.H. Norris, S. Masetto and P. Valli. Dept. of Pharmacol., Tulane Med. Sch., New Orleans, LA 70112 and Ist. Fisiol. Gen. Univ. Pavia, Pavia, Italy.

According to pharmacological theory (Paton, 1961) even antagonists should cause receptor activation, initially, before tight binding occurs. Hanf *et al.* (1993) reported on just such an agonistic effect of atropine (Atr) on muscarinic receptors of cardiac myocytes. The present observation occurred when Atr was used in an attempt to identify the receptor involved in an effect of acetylcholine (ACh) on sensory cells (hair cells) isolated from the frog saccule. The application of Atr (nM-μM) produced changes in currents similar to those produced by ACh in voltage-clamped isolated hair cells. Further, ACh and Atr seemed to be additive in their effects. We then went on to demonstrate that Atr had agonistic effects in vestibular organs *in vitro*. In both semicircular canal and saccule, Atr (pM-nM) facilitated afferent firing rates similarly to ACh. The saccule was significantly more sensitive to Atr's effect than the semicircular canal. Denervation of the efferent, cholinergic innervation of these organs did not alter their responses to Atr. At higher concentrations, Atr has been shown to antagonize the facilitatory effects of ACh in vestibular organs (Housley, *et al.* 1990; Guth *et al.* 1994). Thus, Atr exerts an ACh-like agonistic effect in these organs. Supported by Grant DC 00303 of the N.I.H.

801.12

HEPARIN INTERACTION WITH MUSCARINIC RECEPTORS; SUBTYPE SELECTIVITY AND MECHANISM OF ACTION. Esam E. El-Fakahany*, Shou Zhen Wang and Randee Edmundson. Division of Neuroscience Research in Psychiatry, University of Minnesota Medical School, Minneapolis, MN 55455.

The selectivity of heparin in its positive cooperative effects in influencing binding of antagonist muscarinic receptor ligands was investigated at the five known subtypes of muscarinic receptors. The effects of heparin on binding of [^{3}H]N-methylscopolamine at equilibrium was studied in CHO cells which express each of the individual muscarinic receptor subtypes and in membranes prepared from these cells. Heparin significantly increased equilibrium binding of subsaturating concentrations of the ligand only in membranes of CHO-m2 cells. These effects of heparin were similar to those obtained in cardiac membranes. Heparin did not influence ligand binding to m2 receptors in intact cells, suggesting an action of heparin at a cytoplasmic domain of the receptor or at an intracellular protein which is coupled to m2 muscarinic receptors. The positive cooperative effects of heparin at m2 muscarinic receptors were nearly abolished upon treatment of cells with pertussis toxin. These results suggest that the effects of heparin on ligand binding to m2 muscarinic receptors require an intact interaction between the receptor and G-proteins.

801.13

ANTICHOLINERGIC ANTIPARKINSON DRUGS ARE POTENT ANTAGONISTS OF MUSCARINIC INHIBITION OF D1-STIMULATED ADENYLYL CYCLASE IN RAT STRIATUM. P.Onali* and M.C. Olianas, Dept. of Neurosciences, Univ. of Cagliari, Cagliari, Italy.

Antimuscarinic drugs have long been used for the treatment of Parkinson's disease and extrapyramidal motor disturbances caused by neuroleptics. However, the mechanisms underlying this therapeutic benefit have not been elucidated. Previously, we have reported that in rat striatum muscarinic receptor activation inhibits dopamine (DA) D1 receptor function, as indicated by the reduced DA stimulation of adenylyl cyclase. In the present study we have investigated the ability of various antimuscarinic drugs currently used in the treatment of Parkinson's disease to antagonize the carbachol (CCh) inhibition of adenylyl cyclase activity stimulated by the selective DA D1 agonist (±) SKF 82958. We have found that trihexyphenidyl, benztropine, biperiden, procyclidine and ethopropazine are potent antagonists of CCh inhibitory effect with Ki values ranging from 4.0 to 30 nM. Moreover , there is a good correlation (r = 0.95) between the Ki values and the average clinical doses of the drugs. These data suggest that the removal of a muscarinic inhibitory tone on D1 receptor activity may be one of the mechanisms by which the anticholinergic drugs exert their antiparkinsonian effect.

801.14

STRUTURAL BASIS OF ANTAGONIST BINDING TO m1 AND m2 MUSCARINIC RECEPTORS USING SITE-DIRECTED MUTAGENESIS. M.A.B. TICE*, L.A. TAYLOR, T. HASHEMI AND R.D. McQUADE. CNS Pharmacology, Schering-Plough Research Institute, Kenilworth, NJ 07033

Pharmacological characterization of the muscarinic receptors has been based on affinity for the M1 and M2 selective antagonists, pirenzepine (PZ) and AFDX-116, respectively. The molecular cloning and sequencing of the five muscarinic receptor genes have advanced our ability to investigate receptor binding using such tools as site-directed mutagenesis. In an attempt to map antagonist binding and to probe receptor selectivity, we have performed site-directed mutagenesis on the human m1 and m2 receptor genes. Corresponding sequnces were exchanged between the receptor genes. Mutants were constructed that replaced uncharged residues in m1 (Leu174-Ala175-Gly176-Gln177) with the corresponding charged residues of the m2 sequence (Glu172-Asp173-Gly174-Glu175). Another mutant involved the replacement of the charged Glu397 in m1 with Asn of m2. A similar mutant was constructed in m2 which changed Asn419 to Glu. Radioligand binding studies conducted with these mutants show 2 to 3 fold increase in affinity for PZ by the m2 mutants and a 2 fold increase in affinity of the m1 mutant Asn 397 for the m2 selective antagonist, himbacine.

Site-directed mutagenesis studies performed by Fraser,et al. Mol. Pharm. 36:840(1989) on conserved aspartate residues of the m1 receptor revealed the importance of Asp105 for antagonist binding. We like others have investigated the effect of mutation of conserved amino acids around this pivotal residue. Mutation of the conserved Trp residue in m1 and m2 at positions 101 and 99, respectively, have been constructed. Construction of mutants in the muscarinic receptor family have given insight into the basis of receptor selectivity for various antagonists.

ACETYLCHOLINE RECEPTOR MUSCARINIC: RECEPTORS—EXPRESSION

802.1

***IN SITU* HYBRIDIZATION STUDIES OF THE EXPRESSION OF MUSCARINIC RECEPTOR SUB-TYPE mRNA IN AGED MEMORY-IMPAIRED AND UNIMPAIRED LONG EVANS RATS.** H. Le Jeune*, J-G. Chabot, W. Rowe, M. Meaney and R. Quirion. Douglas Hospital Res. Ctr., Dept. Psychiat. McGill Univ., 6875 Boul. LaSalle, Montreal, Canada H4H 1R3.

We recently reported that the apparent densities of muscarinic M2-like / [^{3}H]AF-DX 384 binding sites are not decreased, but indeed increased, in various cortical and hippocampal areas of 24-25 month-old memory-impaired (AI) vs aged memory-unimpaired (AU) Long Evans rats behaviorally-assessed using the Morris Water Maze (Quirion et al., J. Neurosci., 15:1455, 1995; Aubert et al., Neuroscience, in press). Increases in M2-like receptor binding densities were correlated with altered acetylcholine release, and behavioral improvements were noted following treatment with a potent antagonist, BIBN99 (Quirion et al., ibib). In order to determine if changes in M2-like receptor levels were of genomic/transcriptional nature, the expression of molecularly-defined m1, m2, m3 and m4 receptor mRNAs was studied in the AI/AU model using quantitative *in situ* hybridization and ^{35}S-labeled riboprobes complementary to human muscarinic receptors cDNA sequences (Bonner et al., Science 237:527-532, 1987). Specific mRNA transcripts for each of these four muscarinic receptors are expressed in the rat brain according to a unique profile of distribution globally, but not fully, in accordance with earlier results obtained with oligonucleotides. The apparent level of expression of each mRNA transcript was not significantly different in any regions of the AI vs AU rat brains or between 6-month old Long Evans adult rats and 24-25-month old aged animals. Accordingly, increases in [^{3}H]AF-DX 384 binding sites are not associated with changes in the expression of the m2 and m4 transcripts, the two genes coding for pharmacological-defined M2 receptors recognized by [^{3}H]AF-DX 384. It thus suggests post-transcriptional and/or receptor recycling modifications leading to apparent increases in receptor protein levels in the AI group. Supported by MRCC.

802.2

IN VIVO UPTAKE OF I-123 QNB STEREOISOMERS IN HUMAN BRAIN. R. Coppola*, M.B. Knable, D.W. Jones, J. Gorey, K.S. Lee, D.R. Weinberger. NIMH, IRP, CBDB, 2700 M.L. King Jr. Ave., S.E., Washington, D.C., 20032

I-123 QNB, a radioligand antagonist for muscarinic receptor imaging, has four stereoisomers which have not been fully characterized in vivo in humans. Two normal subjects (aged 34 and 75), underwent SPECT studies with the RS, RR, SR and SS stereoisomers of I-123 QNB in separate scanning sessions. Prolonged time-activity data over a period of 22 hours were obtained . Regions of interest (ROIs) were drawn on MRI scans co-registered with SPECT scans. All isomers produced scans with no detectable binding in the cerebellum. The RS isomer produced scans with the most rapid accumulation of specific binding. The RR isomer produced scans with a slower accumulation and lower peak of specific binding. The SR and SS isomers yielded scans with an early peak of few counts and little anatomic segregation of ligand distribution. The table displays the peak binding for both subjects in cpm/mCi/ml.

	RS		RR		SR		SS	
	young	old	young	old	young	old	young	old
Thalamus	1.4	48.3	5.4	5.5	5.2	6.8	1.4	1.2
Putamen	35.2	139.5	10.1	47.3	4.8	8.8	1.6	1.5
Occ. Ctx.	14.0	134.5	7.2	24.2	3.4	6.3	0.49	0.78

These data, in combination with previous in vitro work (Gibson, J. Nuc. Med., 1984), suggest that the RS and RR isomers have different affinities at the M1 and M2 receptor subtypes that may be discernible in vivo. Also, there appears to be an age related increase in the number of unoccupied M1 and M2 receptor subtypes.

802.3

SUBTYPE-SPECIFIC REGULATION OF MUSCARINIC RECEPTOR EXPRESSION AND FUNCTION BY HETEROLOGOUS RECEPTOR ACTIVATION. D. A. Jackson* and N. M. Nathanson, Department of Pharmacology, University of Washington, Seattle, Washington 98195.

Incubation of cultured embryonic chicken heart cells with the β-adrenergic agonist isoproterenol resulted in a dose-dependent increase in the number of mAChR on the surface of intact cells. The isoproterenol-mediated increase in mAChR number was time-dependent and reached a maximum by 48 hours. Chick heart cells treated with isoproterenol exhibited a greater than 6 fold increase in the sensitivity for carbachol-mediated inhibition of adenylyl cyclase activity as compared to control. Stimulation of cultured heart cells for 24 hours with isoproterenol resulted in a 25-35 % increase in cm2 mRNA levels as compared to control cm2 mRNA levels. In contrast, the level of cm4 mRNA was not significantly affected by isoproterenol treatment. cm2 mRNA levels were maximally elevated by fifteen hours following isoproterenol stimulation and remained elevated for up to 72 hours. Incubation of cells with isoproterenol in the presence of Rp-cAMP, an inhibitor of cAMP-dependent protein kinase, blocked the increase in the level of cm2 mRNA. Thus, prolonged activation of β-adrenergic receptor results in an increase in mAChR number and muscarinic responsiveness in chick heart cells due to a cAMP-dependent protein kinase mediated increase in cm2 mRNA levels.

802.4

LOCALIZATION OF MUSCARINIC ACETYLCHOLINE RECEPTOR SUBTYPES IN HUMAN BRAIN USING BY SUBTYPE SPECIFIC ANTIBODIES. Shiozaki K.[1], Uchiyama H.[2] and Nakata H.*[3] [1] Fukushimura Hospital, 19-14 Yamanaka Noyoricho Toyohashi 441 Japan [2]Dept. of Neurosurgery, Hamamatsu Redcross Hospital [3]Dept. of Pharmacology, National Defence Medical College.

We have attempted to generate subtype specific antibodies against muscarinic acetylcholine receptor subtypes(mAChR, m1-m4) and to examine their distributions in human brain. Large regions of the third cytoplasmic loops(i3) of the porcine(m1, m2) and rat(m3,m4) receptors were expressed as fusion proteins and used for immunization. Each antiserum immunoprecipitated each subtype of mAChR expressed in Sf-9(m1, m2 and m4) or CHO(m3) cells in a subtype-specific manner. Membrane fractions were prepared from postmortem human brains(80 year old male, 85 year old female, 91 year old male), histopathologically diagnosed as normal aging. Concentrations of mAChRs in different regions of the brain of these preparations as determined by ^{3}[H]QNB binding were 1250±66(frontal cortex), 1028±159 (hippocampus), 1685±329(caudate nucleus) and 984±119(thalamus) f mol /mg protein. mAChRs were prelabeled with ^{3}[H]QNB, then solubilized with 1% digitonin and 0.1% cholate and immunoprecipitated with antisera. The proportions of specifically precipitated mAChR subtypes were estimated as the differences in the amounts of bound ^{3}[H]QNB precipitated with specific antibody and those precipitated with non immune serum. The estimated percentages were as follows:frontal cortex (m1:38.1±1.3, m2:9.1±0.9, m3:6.3±1.1, m4:5.8±3.3), hippocampus(m1:40.2±2.4, m2:9.0±0.3, m3:5.6±0.6, m4:4.9±2.7), caudate nucleus(m1:43.7±2.4, m2:12.5±2.4, m3:4.1±0.9, m4:7.0±3.1) and thalamus(m1:3.6±6.4, m2:46.0±6.8, m3:4.6±2.8, m4:-1.3±0.8).

802.5

EXPRESSION OF MUSCARINIC M3 RECEPTORS mRNA IN HUMAN BRAIN MICROVASCULAR FRACTIONS, ASTROCYTES AND VASCULAR CELLS IN CULTURE. D.G. Linville*[1], Z. Cohen[2], A. Olivier[2], W.V. Yong[2], D. Stanimirovic[3], and E. Hamel[2]. [1]Mercyhurst Coll, Erie, PA, 16546 USA, [2]Montreal Neurological Institute, McGill Univ, Montréal, QC, Canada H3A 2B4 and [3]National Research Council of Canada, Ottawa, ON, Canada.

Subtype specific muscarinic acetylcholine receptors (mAChR) have been identified pharmacologically in human isolated cerebral microvessels (MVs) and capillaries (CAPs), of which the M3 subtype is thought to mediate relaxation (Linville & Hamel, *Naunyn-Schmied. Arch. Pharmacol.*, in press). We investigated the expression of m3 mAChR mRNA by reverse-transcriptase/polymerase chain reaction (RT/PCR) and/or IP_3 production in isolated human cortical MVs and CAPs obtained post-mortem, astrocytes cultured from human fetal brain (HFBA) and subcloned endothelial (HBEC) or primary cultures of smooth muscle (HBSM) cells. The latter were respectively dissociated from CAPs (< 112μm) or MVs (> 112 or > 350μm) isolated from human temporal lobe excised at surgery for the treatment of epilepsy. MVs, CAPs and HBEC were all enriched in γ-glutamyl transpeptidase and alkaline phosphatase. HBEC and HFBA were respectively and selectively immunopositive for Factor-VIII and GFAP. Gel electrophoresis of the PCR products generated with specific m3 mAChR oligonucleotide primers yielded a band of the expected size in preparations of human MVs (n=3), CAPs (n=3), HBFA (n=3), HBSM (n=2), but not HBEC (n=4). However, mAChR agonist bethanechol (1mM) moderately increased (~70%) IP_3 production in HBEC. These results show that m3 mAChR mRNA is expressed in freshly isolated human cortical MVs, CAPs as well as in astrocytes and primary cultures of smooth muscle cells, and that HBEC express mAChRs couple to phospholipase C activation. Further work is needed to explain to apparent discrepancy between HBEC and freshly isolated CAPs (mainly enriched in endothelial cells) in m3 mAChR expression. Supported by MRC and NRC of Canada and a McGill major studentship (Z.C.).

802.6

OXIDATIVE BURST IN NEURONAL CELLS AFTER MUSCARINIC STIMULATION. J. Naarala*, P. Tervo, J. Loikkanen and K. Savolainen[1]. National Public Health Institute, Department of Toxicology, P.O.B. 95, FIN-70701 Kuopio, and [1]Department of Environmental Sciences, University of Kuopio, Finland.

The effects of a muscarinic receptor agonist, carbachol (CCh), on the production of reactive oxygen metabolites (ROM) and the levels of the intracellular glutathione (GSH) were studied in human SH-SY5Y neuroblastoma cell line. ROM and GSH were measured by using fluorescent probes, dichlorofluorescin (DCF) and monochlorobimane (MBCL), respectively. The translocation of protein kinase C (PKC) to the cell membrane was measured by phorbol dibutyrate (PDBu) binding and muscarinic receptor number was assayed by using quinuclidinylbenzilate (QNB) binding in intact SH-SY5Y cells. One millimolar CCh increased ROM production 1.87 fold at 60 min, 2.37 fold at 120 min, and 3.0 fold at 180 min as compared to the control values. However, CCh, at a concentration of 500 μM, caused only a slight increase in ROM production, and values obtained with 100 μM CCh remained at control levels. Intracellular GSH levels decreased by about 25% when the cells were incubated for 120 min with 1 mM CCh. However, GSH levels returned back to the control level after 180 min incubation. PDBu binding (B_{MAX}) increased by 73% after 20 min incubation with 1 mM CCh as compared to the control cells. Also 500 μM CCh increased in PDBu binding 33-54% as compared to corresponding controls, i.e. PKC translocation to the membrane was enhanced. QNB binding (B_{MAX}) increased by 166% after 60 min incubation with 1 mM CCh, and returned back to the control level after 180 min incubation. We conclude that muscarinic receptor stimulation with CCh may cause oxidative stress in human neuroblastoma cells. Oxidative burst in neuronal cells may be partially routed through PKC, because increase in PDBu binding is a prerequisite for the production of ROM. However, there may be other effector mechanisms that have to be activated prior to the production of ROM. Supported by the Academy of Finland.

802.7

MUSCARINIC ACETYLCHOLINE RECEPTOR EXPRESSION IN DIABETIC RAT BRAIN. M.E. Morton*, T.N.Clancy, D.W. Laidlaw, and C.E. Sanz, Department of Biology, College of the Holy Cross, Worcester, MA 01610.

Current evidence suggests that neuropathies are a common complication associated with human insulin-dependent diabetes mellitus (IDDM). Although structural and functional changes occur in neural tissues in individuals with diabetes, the molecular mechanisms responsible for aberrant neural function and structure in diabetes are not understood. The BB diabetic rat is an appealing system in which to study these mechanisms since the BB rat model closely resembles human IDDM. The muscarinic acetylcholine receptor (mAChR) is the major receptor for acetylcholine in the brain and its expression has been shown previously to be affected by insulin. Therefore, the neuronal mAChR pathway is most likely affected in diabetes. To investigate the expression of the mAChR and downstream targets in diabetes in order to begin to dissect the role of altered mAChR function in the development of diabetic neuropathies, [^{3}H]QNB binding analysis was performed on brain tissue homogenates from male diabetic BB rats vs. age- and sex-matched control rats. Diabetic rats were provided with insulin up to 30 hours prior to analysis. The data demonstrate a two-fold increase in expression of mAChR in diabetic rat brain This change is specific for mAChR expression and not downstream components of the signaling pathway since neither G-protein (Gβ, Giα1, Giα2, Giα3, Gs, or Go) nor voltage-gated calcium channel (α2 subunit) expression is altered in the hippocampus, cortex, brainstem, or cerebellum of diabetic rats. Supported by a grant from NIH (NINDS) #31757-01.

802.8

ACETYLCHOLINE (ACh) STIMULATES THE RELEASE OF NITRIC OXIDE FROM RAT SPINAL CORD IN VITRO. Z Xu, C Tong, JC Eisenach*, P Li, The Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1009

Previous in vivo studies suggest that Nitric Oxide(NO) may mediate the actions of ACh on spinal sympathetic neuronal activity and on antinociception in the spinal cord dorsal horn. In this study we utilized a novel bioassay for NO release to examine the effect of ACh perfusion of rat spinal cord in vitro on NO release. Following Animal Care Committee approval, adult male Sprague-Dawley rats were euthanized, the spinal cord removed, and spinal cord slices placed in tissue chambers perfused continuously with oxygenated Krebs solution which exited the chamber to fall on endothelium denuded aortic rings. Successful removal of endothelium was tested by lack of relaxation to ACh. After preconstriction with phenylephrine, ACh (10^{-12} to 10^{-4} M) alone or with other drugs (all 10^{-5} M) were added into the perfusion solution, with continuous recording of the detector ring's tension. Data are expressed as % relaxation (mean ± SEM), and were analyzed by 2-way ANOVA on the full dose response curves, with $P < 0.05$ considered significant. Perfusion directly on detector rings of ACh caused no change in their tension, whereas perfusion over rat spinal cord tissue with ACh resulted in a dose-dependent relaxation of the detector ring, with maximum relaxation of 55%. This relaxation was blocked by n-methyl-l-arginine (NMLA), hemoglobin, or methylene blue. ACh-induced relaxation was also blocked similarly by the muscarinic antagonists, atropine, pirenzepine, or AFDX-116. This study showed that ACh causes release of a vasodilator from spinal cord slices in vitro which shares the pharmacology of NO (active on endothelium-denuded rings, blocked by the NO synthase inhibitor, NMLA, the NO scavenger, hemoglobin, and the guanylate cyclase inhibitor, methylene blue), and involving both M1 and M2 receptors activation.
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802.9

EFFECT OF FORSKOLIN ON CARBACHOL-INDUCED SIGNAL TRANSDUCTION IN CANINE CULTURED TRACHEAL SMOOTH MUSCLE CELLS. C. M. Yang*. Department of Pharmacology, Chang Gung College of Medicine and Technology, Kwei-San, Tao-Yuan, Taiwan.

The effect of elevating cyclic AMP on carbachol-induced generation of inositol phosphates (IPs) and rise in intracellular Ca^{2+} ($[Ca^{2+}]i$) was investigated in canine cultured tracheal smooth muscle cells (TSMCs). Pretreatment of TSMCs with either cholera toxin, forskolin, or dibutyryl cyclic AMP inhibited carbachol-stimulated Ca^{2+} mobilization and IPs accumulation. The inhibitory effects of these agents produced both depression of the maximal response and a shift to the right of the concentration-response curve of carbachol without changing the EC_{50} values. Even after treatment with forskolin for 24 h, the cells retained the ability to respond to carbachol-induced Ca^{2+} mobilization to the same extent as the control group. The K_b and Bmax values of the muscarinic receptor (mAChR) for [^{3}H]N-methyl scopolamine binding were not significantly changed by forskolin treatment, suggesting that the inhibitory effect of forskolin is distal to the mAChR. The AlF_4^--induced IPs accumulation was inhibited by forskolin, supporting that G protein(s) are directly activated by AlF_4^- and uncoupled to phospholipase C by forskolin treatment. We conclude that cyclic AMP elevating agents inhibit carbachol-stimulated responses in TSMCs. Since generation of IPs and increase in $[Ca^{2+}]i$ are very early events in the activation of mAChRs, attenuation of these events by cyclic AMP elevating agents might well contribute to the inhibitory effect of cyclic AMP on tracheal smooth muscle function.

802.10

Hippocampal Sympathetic Ingrowth and Cholinergic Denervation Differentially Alter Hippocampal Muscarinic Receptors Over Time. M.R. Roberson, K. Kolasa, D.S. Parsons, L.E. Harrell*. Alzheimer's Disease Center, Depts. of Psychology and Neurology, VA and Univ. Alabama Med. Ctr., Birmingham, AL 35294

Degeneration of forebrain cholinergic neurons and hippocampal neuronal reorganizations both occur in Alzheimer's Disease (AD). Our laboratory has been utilizing the model of hippocampal sympathetic ingrowth (HSI), which has been suggested to occur in AD, to investigate the functional effects of cholinergic denervation (CD) and hippocampal rearrangements. In this model, peripheral sympathetic fibers, originating from the superior cervical ganglia, grow into the hippocampus, after cholinergic denervation via medial septal lesions (MSL). In this study we examined the long-term effects of these treatments on muscarinic cholinergic receptors (mAChR), by examining [^{3}H]-QNB, a nonselective mAChR antagonist, binding in hippocampal membranes 3 months post lesion. Four groups of animals were employed: Controls (sham MSL + sham ganglionectomy (Gx); HSI (MSL + sham Gx); CD (MSL + Gx); Gx (sham MSL + Gx). In dorsal hippocampal membranes, binding affinity (K_D) was found to be significantly decreased in HSI ($p < .002$) and CD ($p < .01$) groups as compared to controls, while number of mAChR (Bmax) was found to be significantly increased in HSI as compared to controls ($p < .02$) and CD ($p < .01$) groups. These results suggest that long-term cholinergic denervation (found in both HSI and CD groups) decreases mAChR affinity, while the presence of sympathetic fibers increases the number of mAChR. Thus, both HSI and cholinergic denervation affect mAChR, a bit differently. Further, these results may provide some insight into why cholinomimetic therapies are ineffective for most AD patients, in that a decrease in mAChR affinity would reduce agonist efficacy at the mAChR.

802.11

EFFECT OF (±)-EPIBATIDINE ON THE RELEASE OF CATECHOLAMINES: BIOCHEMICAL AND BEHAVIORAL EVIDENCE IN RATS. A.I. Sacaan*, F. Menzaghi, J.L. Dunlop, L.C. Correa, K.T. Whelan, and G.K. Lloyd
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The present study determined the effect of (±)-epibatidine (Epi), a neuronal nicotinic acetylcholine receptor (NAChR) agonist on catecholamine release. (±)-Epi (3-300 nM) produced a concentration-dependent increase in [^{3}H]-dopamine release from rat striatal slices, and [^{3}H]-norepinephrine release from rat hippocampal and thalamic slices, with differential sensitivity to various NAChR antagonists as shown below:

Tissue	EC_{50} (nM)	Mec 3 (uM)	d-TC 100 (uM)	DHβE 100 (uM)	TTX 1 (uM)
Striatum	37 ±10	+	-	+	+
Hippocampus	20 ±2.6	+	+	-	+
Thalamus	23 ±5.3	+	+	-	+

In addition, (±)-Epi (1-3 ug/kg, s.c.) and nicotine (350 ug/kg, s.c.) produced ipsilateral turning in the unilaterally 6-hydroxy-dopamine lesioned rat, consistent with the stimulation of dopamine release from the intact nigrostriatal dopamine pathway. This effect was blocked by mecamylamine (mec) (3 mg/kg, s.c.). Moreover, (±)-Epi (1-3 ug/kg, s.c.) increased locomotor activity in a dose-dependent manner. This effect was blocked by mec as well as the D1 and D2 receptor antagonists, SCH23390 and eticlopride, respectively. These results demonstrate that, in addition to the previously reported analgesic activity, (±)-Epi displays NAChR agonist activity in the rat central nervous system and that certain effects may be mediated via NAChR-stimulated catecholamine release, and subsequent activation of corresponding receptors.

802.12

CONSTITUTIVE ACTIVATION OF MUSCARINIC RECEPTORS BY THE G-PROTEIN G_q E. S. Burstein*, T. A. Spalding, H. Bräuner-Osborne, M. R. Brann, Department of Psychiatry, University of Vermont, Burlington VT 05405 and Receptor Technologies, Winooski, VT 05404.

In the absence of ligands, G-protein coupled receptors interconvert between active and inactive conformations. These conformations are stabilized by agonists and antagonists, respectively. Like agonists, G-proteins are thought to preferentially associate with receptors in the active conformation and should therefore be able to promote their formation in the absence of agonist. We show that overexpression of $G_{\alpha Q}$, but not $G_{\alpha 12}$ induces constitutive activation of compatible (m1, m3, and m5 but not m2) muscarinic receptors and this activity is blocked by muscarinic antagonists. Gq also increases the potency and efficacy of agonists. The phenotypes of receptors activated by G-proteins are very similar to the phenotypes of mutationally activated receptors. These results indicate that regulation of G-protein levels has a profound impact on receptor control of cellular physiology, even in the absence of agonist ligands.

802.13

G PROTEIN βγ SUBUNITS MIMIC THE MUSCARINIC REGULATION OF ADENYLYL CYCLASE OF RAT OLFACTORY BULB. M.C. Olianas*[1], H. Hamm[2] and P. Onali[1], Dept. of Neurosciences, University of Cagliari, Italy [1] and Dept. Physiol. and Biophys., Univ. of Chicago, IL 60680[2].

In membranes of rat olfactory bulb activation of muscarinic receptors enhances basal and neurotransmitter-stimulated adenylyl cyclase activities but inhibits Ca^{2+}/calmodulin and forskolin (FSK) stimulations of the enzyme. In the present study we show that this bimodal control of cyclic AMP formation can be reproduced by the G protein βγ subunits. Thus, incubation of olfactory bulb membranes with the βγ subunits of transducin elicited a concentration-dependent increase of basal adenylyl cyclase activity with an EC50 of about 100 nM. The βγ stimulatory effect was not additive with that produced by maximal activation of muscarinic receptors. Moreover, as observed with muscarinic receptor agonists, the βγ subunits significantly potentiated the stimulation of cyclic AMP formation by vasoactive intestinal peptide and inhibited the adenylyl cyclase activity stimulated by FSK with an IC50 of about 40 nM. These data support the idea that in rat olfactory bulb muscarinic receptors exert a bimodal control on cyclic AMP formation by promoting the release of G protein βγ subunits which then differentially affect the various molecular forms of adenylyl cyclase.

803.1

HYDROGEN PEROXIDE AND HYDROXYL RADICAL, BUT NOT SUPEROXIDE, PROMOTE A CONCENTRATION-DEPENDENT INHIBITION OF GLUTAMATE UPTAKE BY ASTROCYTES. O. Sorg and F.E. Bloom*. Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Glutamate uptake (Glu up) by astrocytes is a very important function which prevents the potential neurotoxicity of this neurotransmitter after its release by neurons. Recently, Volterra et al. showed that H_2O_2 induced an inhibition of Glu-up by primary astrocytes in cultures. Using the same model, we tried to define the role of three main reactive oxygen species in Glu up inhibition, as well as the efficiency of endogenous protection mechanisms. H_2O_2 promotes an inhibition of Glu up in a concentration-dependent manner. O_2-, by itself, has no effect, suggesting that the reactive species in the xanthine/xanthine oxidase system is a combination of H_2O_2 and ·OH radical produced by the Haber-Weiß reaction. The Glu up inhibitory effect of H_2O_2 is potentiated by adding CuSO4 and ascorbate - a condition known to produce ·OH radical - or N-ethylmaleimide. The latter compound could act as a thiol-modifier group of the glutamate transporter and/or as a glutathione peroxidase I inhibitor; these possibilities are under investigation. (Supported by MH 47680).

803.2

INCREASED GLUTAMATE UPTAKE BY GLIAL CELLS DURING ATP DEPLETION IN HIPPOCAMPAL SLICES. J.E. Madl*. Department of Anatomy & Neurobiology, CSU, Ft. Collins, CO 80523.

Diverse metabolic insults can cause neuronal damage via the release of excitatory amino acids (EAAs), including glutamate (Glu). Severe ATP depletion releases EAAs by reversal of cotransporters that normally act to clear the synapse of transmitter EAAs. However, many metabolic insults produce only mild to moderate ATP depletion. Therefore, the effects of moderate ATP depletion on EAA uptake were determined. Uptake of the EAA analog D,L threo-b-hydroxyaspartate (OH-Asp) was used as an indicator of Glu uptake. OH-Asp uptake and the ATP content of rat hippocampal slices were measured by HPLC. Moderate ATP depletion (80% ≥ ATP ≥ 40% of control) increased uptake of OH-Asp. In contrast, more severe ATP depletion (ATP<40% of control) decreased OH-Asp uptake and increased release of endogenous Glu, suggesting reversal of cotransporters. Selective depletion of ATP from glia using fluoroacetate and fluorocitrate resulted in a similar increase in uptake of OH-Asp, suggesting that much of the increased uptake occurred in glial cells. Immunocytochemical localization of Glu in metabolically insulted slices indicated that Glu was decreased in nerve terminal-like puncta and increased in cells with glial-like morphology, suggesting that glial cells accumulate EAAs released by neurons. Increasing extracellular phosphate blocked the increased uptake of EAAs, suggesting that the increases of intracellular phosphate induced by ATP depletion may increase the phosphate gradient and potentiate EAA uptake. The increased uptake of EAAs during mild to moderate ATP depletion suggests that during many moderate insults, such as partial ischemia, a decrease in extracellular Glu might contribute to loss of CNS function.

803.3

GLUTAMATE UPTAKE BY HIPPOCAMPAL ASTROCYTES IS INHIBITED BY FERROUS CHLORIDE. J. H. Pizzonia*, E. O'Conner and D. L. Kraemer. Dept. of Surgery, (Neurosurgery), Yale University School of Medicine, New Haven, CT. 06520

Iron-complexed to blood breakdown products has been implicated in the pathogenesis of stroke and reperfusion injury, trauma and epilepsy. Excessive accumulation of iron during aging has also been implicated in several neurodegenerative diseases. We hypothesized that iron may affect neuronal injury by inhibiting glutamate transport mechanisms. As astroglial regulation of glutamate is primarily responsible for modulated synaptic glutamate activity, we examined the effect of iron on glutamate uptake and efflux in astrocytes. Hippocampal astrocytes were prepared from neonatal rats and preincubated in HEPES buffer containing 50 μM ferrous chloride for 30 minutes before the addition of ^{3}H-glutamate. Uptake was determined from 2-60 minutes and total radioactivity determined from cell lysates. Ferrous chloride decreased glutamate uptake by 50% at 10 minutes (n=4, p<.05), and by 70% after 60 minutes (n=4, p<.001) compared to control cells incubated in HEPES buffer without iron. In separate experiments the rate of ^{3}H-glutamate efflux was not changed by addition of 50 μM ferrous chloride. Preincubation with the calcium channel blocker, lanthanum chloride, (1 μM) did prevent inhibition of ^{3}H-glutamate uptake by 50 μM ferrous chloride. We conclude that iron inhibits glutamate uptake into astrocytes and thereby may induce neurotoxicity by a mechanism independent of transmembrane calcium influx.

803.4

EXCITOTOXICITY BY TRANSPORTER-MEDIATED GLUTAMATE EFFLUX FROM NEURONS AND GLIA IN NON-ISCHEMIC CONDITIONS A. Volterra*, P.Bezzi, B. Lodi Rizzini, D. Trotti & G. Racagni Ctr Neuropharmacol, Inst Pharmacol Sci, Univ Milan, 20133, Italy

Uptake in neurons and astrocytes by distinct transporter subtypes keeps low (≤1 μM) extracellular glutamate concentration ($[GLU]_o$) with crucial implications for excitatory synaptic transmission. In ischemia, loss of the physiological ion gradients causes transporters to function in reversed mode pumping out GLU up to neurotoxic levels. We have reported that addition of a competitive GLU uptake inhibitor, t-pyrrolidine-2,4-dicarboxylate (PDC, 200 μM, 30' in normal salt buffer with 1 mM Mg^{++}) to cortical neuroglial co-cultures, induces rapid increase of cell-released $[GLU]_o$ (from 1 to 5 μM) and, as a consequence, delayed (1 day) neurotoxicity (Volterra et al., Soc.Neurosci.Abs.20:271,1994). Toxicity is prevented by the NMDA-antagonist APV (100 μM) and attenuated (40-60%) by AMPA/kainate (CNQX, 10 μM) and metabotropic (MCPG, 500 μM) blockers. Surprisingly, both $[GLU]_o$ increase and neurotoxicity with PDC are largely (70%) unaffected by 1 μM TTX, despite that TTX abolishes excitatory synaptic activity in the culture, as recorded by whole-cell current-clamped neurons. However, in the presence of TTX, we observe GLU release from astrocytes of sister glial cultures in response to PDC (200 μM, 30'), an effect abolished by replacement of Na^+_o with Li^+. Moreover, Li^+ substitution reduces by >80% the TTX-insensitive component of $[GLU]_o$ increase observed in co-cultures. Therefore, our data indicate that: (1) transporter-mediated (Li^+-sensitive) GLU efflux from both glia and neurons, in addition to reduced reuptake of synaptically released GLU, accounts for PDC-induced $[GLU]_o$ rise and neurotoxicity; (2) reversed GLU transport function can be activated even under normal, non-ischemic conditions (e.g. via heteroexchange) with direct neuroexcitotoxic consequences.

803.5

HUMAN NERVE TISSUE-SPECIFIC GLUTAMATE DEHYDROGENASE ADAPTED TO FUNCTION UNDER LOW ENERGY STATES AND INCREASED GLUTAMATE RELEASE. P. Shashidharan*, Donald D. Clarke[1], and Andreas Plaitakis. Department of Neurology, Mount Sinai School of Medicine, One Gustave L Levy Place, New York, N.Y., 10029, [1]Dept. of Chemistry, Fordham University, Bronx, New York.

Human glutamate dehydrogenase (GDH) exists in nerve tissue-specific and housekeeping isoforms encoded by distinct genes. We have obtained both these proteins in pure form by expressing the corresponding cDNAs in Sf9 cells using the baculovirus expression system. The specific expression of each GDH isoproteins by the corresponding cell lines was verified by the N-terminal amino acid sequencing. Heat inactivation studies revealed that the nerve-tissue specific GDH was heat-labile and the housekeeping enzyme heat stable. In the absence of allosteric effectors, the two isoenzymes differed markedly in their ability to interconvert glutamate to α-ketoglutarate. The nerve-specific enzyme was largely inactive whereas the housekeeping GDH showed 40-50% of its maximal activity. ADP induced a concentration-dependent (10-1,000 μM) activation that was proportionally greater (200 - 6,000%) for the nerve tissue-specific than the housekeeping GDH (50-260%). GTP, known to be present in brain at relatively high levels, inhibited the housekeeping GDH but had little effect on the nerve tissue-specific enzyme. Since GDH is highly concentrated in synaptic astrocytes where it is involved in transmitter glutamate metabolism, these allosteric properties evidently represent an adaptation enabling the brain-specific GDH to oxidize increased loads of transmitter glutamate upon extensive hydrolysis of ATP to ADP that may occur in energy failure and/or increased glutamate release.

803.6

REGULATION AND PATHOLOGY OF KYNURENINE PATHWAY METABOLISM IN RAT BRAIN. J. Luthman*, Eva Vänerman, Göran Fredriksson and Bodil Fornstedt-Wallin. Astra Arcus AB, Södertälje, Sweden.

The kynurenine pathway, which is the principal route of hepatic L-tryptophan (TRY) metabolism, also appears to play an important role in brain function. A kynurenine pathway metabolite of particular interest in this regard is quinolinic acid (QUIN), which acts as a NMDA receptor agonist and excitotoxin. Endogenous QUIN and its biosynthetic enzyme 3-hydroxyanthranilic acid dioxygenase (3-HAO) occur in the brain and QUIN levels have been shown to increase in various neurodegenerative processes. In cultures of rat brain tissue, exposure to QUIN and the precursor 3-hydroxyanthranilic acid (3-HANA) produce neuronal loss. Furthermore, administration of QUIN or the precursor *in vivo* to rats leads to cerebral damage. However, the knowledge about the relative contribution of peripheral and central kynurenine pathway enzymes in the *in vivo* regulation of cerebral QUIN is limited. In Sprague-Dawley rats, intracerebroventricular (i.c.v.) administration of 3-HANA, but not other precursors of QUIN, induced a major increase in hippocampal QUIN, that was saturable at high doses and peaked at 2h after administration. Co-administration with a 3-HAO inhibitor blocked the 3-HANA-induced increase in QUIN. Systemic administration of tryptophan, 3-HANA and QUIN itself increased cerebral QUIN, while much smaller effects were seen after administration of L-kynurenine and 3-hydroxykynurenine. Systemic, but not i.c.v., administration of the 3-HAO inhibitor partially counteracted the increase of cerebral QUIN after systemic TRY or 3-HANA. However, in animals with brain damage, such as ischemia and neurotoxin-induced lesions, i.c.v. administration of the inhibitor decreased cerebral QUIN. This indicates that, although cerebral QUIN levels are regulated by the availability of precursors in the periphery, cerebral synthesis plays a significant role when central QUIN levels are enhanced under pathological conditions.

803.7

INTRACEREBRAL GLUTAMATE AND CEREBRAL BLOOD FLOW DURING CHRONIC HYPOXEMIA IN THE NEAR-TERM OVINE FETUS

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Using chronic *in utero* microdialysis (ref.), we examined the relationship between change in cerebral blood flow (CBF) and glutamate (Glu) efflux from the parasaggital parietal cortex (PSPC) in the near term ovine fetus during chronic fetal hypoxemia.

Six near-term ovine fetuses (2 control, 4 experimental) were chronically instrumented with vascular catheters and a microdialysis probe inserted in the PSPC. An adjustable vascular occluder was placed around the common uterine artery. After a 3 day recovery and a 24 hour probe equilibration period the vascular occluder was tightened in experimental animals to reduce the fetal 0_2 saturation by 50% for a 24 hour period without progressive metabolic acidemia. Fetal CBF was determined by injection of radiolabelled microspheres at 0, 8, 16 and 24 hours . Microdialysate samples were collected at 28 minute intervals and the concentration of Glu determined by HPLC.

Basal Glu efflux was 102 ± 64 ng/ml and basal CBF was 227 ± 61 ml/100 g · min. In 3 of 4 experimental animals there was an increase in CBF and Glu efflux in the PSPC while in 1 experimental animal and both controls the CBF and Glu were unchanged. All fetuses experienced an increase in Glu efflux post mortem.

In this preliminary study, ovine fetuses responding to hypoxemia by increasing CBF also show an increase in PSPC Glu efflux. Glu did not increase in animals that failed to show an increase in CBF.

Reference: Penning et al Anesthesiol:82 (1995), p 521-30

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803.8

LONG-TERM CHANGES IN BRAIN FOLLOWING CONTINUOUS PHENCYCLIDINE ADMINISTRATION: STUDIES USING 2-FDG, FLUNITRAZEPAM, KETANSERIN, MAZINDOL, QNB, AND LIGANDS FOR AMPA AND PCP RECEPTORS. G. D. Ellison* and A. KEYS. Department of Psychology, UCLA, 405 Hilgard Ave., Los Angeles, CA 90024

When given continuously for several days, NMDA antagonists such as phencyclidine (PCP) and dizocilpine (MK-801) induce neural degeneration in a variety of limbic structures such as retrosplenial cortex (RSCx), entorhinal cortex (ENTCx), dentate of hippocampus (DEN), and olfactory regions. This has been demonstrated using a variety of measures, including silver-stains. Further autoradiographic studies were conducted in animals at both 24 hours and 21 days after binge PCP administration using 2-FDG and a variety of receptor ligands.

24 hours after pellet removal there were still large increases in glucose uptake in these same limbic structures, and many of these regions were still present after 21 days of recovery. These alterations were accompanied by decreased flunitrazepam but increased mazindol binding in many of these same limbic regions. QNB and AMPA binding was decreased but TCP binding increased in many brain regions, including striatum. These studies may indicate an anatomical substrate for the persisting psychoses which sometimes occur following PCP.

803.9

HIPPOCAMPAL EXCITATION AND TOXICITY PRODUCED *IN VIVO* BY DOMOIC ACID. *P.M. Gross*[1,5] T.M. Polischuk,[2] D.S. Wainman[1], & D.F. Weaver[3,4]*
Depts. [1]Surgery (Neurosurgery), [2]Anatomy & Cell Biology, [3]Chemistry, [4]Medicine (Neurology), & [5]Physiology, Queen's Univ. and Kingston Gen. Hospital, Kingston, Ont., Canada K7L 3N6.

Excitotoxic processes are implicated in many conditions of neuronal damage, including Alzheimer's and Huntington's disease, epilepsy, traumatic head injury, and stroke. Domoic acid (DOM), a glutamate analog from seaweed that contaminates shellfish, is a potent neurotoxin that putatively stimulates glutamate AMPA/kainate receptors (T.M. Polischuk and R.D. Andrew, this meeting). Microinjection of DOM (25-100 pmol) into the lateral cerebral ventricle of conscious rats produced convulsive signs such as tonic tail and forelimb extension, forepaw flexion, exophthalmos, hypoactivity (unresponsiveness), and hyperventilation. Within 5-20 min, DOM evoked increases in mean arterial pressure, plasma glucose levels, and heart rate. Using the quantitative autoradiographic [^{14}C]deoxyglucose technique to measure rates of tissue glucose metabolism, we determined the neuroexcitatory responses of various brain regions to DOM at 4 doses: 100, 50, 25, and 0 pmol. Subregions of the hippocampal formation, ipsilateral (but not contralateral) to the injection site, had dose-dependent, elevated rates of glucose metabolism, including Ammon's horn (+68% in CA1, +130% in CA2, and +194% in CA3), dentate gyrus (+46%), and the fimbria (+49%) (mean responses to 100 pmol DOM are shown). Upon histological inspection by light microscopy, we found that neurons in the pyramidal layer of CA3 ipsilateral to injection were hyperchromatic, irregularly shaped, and surrounded by enlarged extracellular spaces. These findings are pathological signs consistent with excitotoxic cell damage. The caudate, lateral septal, and septofimbrial nuclei also displayed substantial metabolic stimulation near the injection site (>100% increase in ipsilateral metabolic rate). Together, the metabolic and histological results reveal that DOM is a potent, dose-dependent neuroexcitant and toxin in the intact brain.

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803.10

DIFFERENTIAL SUSCEPTIBILITY TO DOMOIC ACID INDUCED TOXICITY IN THE DEVELOPING RAT: A BEHAVIOURAL STUDY. S.M. Strain, G.V. Allen* and R.A.R. Tasker*. Dept. Anat. & Physiol., Atlantic Vet. College, UPEI, Charlottetown, PEI, C1A 4P3 and *Dept. Anat. & Neurobiology, Dalhousie Univ, Halifax, NS B3H 4H7, Canada

Domoic acid (DOM) is an excitatory amino acid that has been identified as a potent neurotoxin in both people and experimental animals. The effects of DOM in the mature brain have been well documented but little information is available concerning its action in the immature brain. This study was designed to assess the toxic effects of systemically administered domoic acid in developing male and female Sprague-Dawley rats. Intraperitoneal injections of five doses (n=6 for each) of domoic acid or saline were administered on postnatal days 0, 5, 14, 22, 30 (f) and 50 (m). Dosages ranged from (0.05 - 2.0 mg/kg) depending on the developmental age. Each animal's behaviour was recorded each minute for 120 minutes post injection using a 3 point scale of increasing severity. Dose response curves as a function of age were generated and statistically compared for parallelism and potency. Prior to weaning pups were found to be significantly more susceptible to domoate induced toxicity, as indicated by both increased potency and the occurrence of convulsive behaviours involving both fore and hind limbs. Post-weaning the more classic limbic seizure was observed and was characterized by the occurrence of forelimb tremors in a reared position and masticatory movements. Data indicate that susceptibility to domoic acid toxicity in the developing rat may be dependent upon establishment and maturation of limbic system circuitry.

803.11

Neuropathology of Subchronic Oral Administration of Domoic Acid (DA) in the Rat. **O.Pulido*;R.Mueller;J.Truelove;F.Iverson;P.Rowsell and M.Bujaki.**
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Amnesic shellfish poisoning (ASP) is caused by eating shellfish containing elevated levels of DA. This study aimed at determining the effect of repeated consumption of DA. Male and female Sprague-Dawley rats were dosed by gavage for 64 days with 0, 0.1 or 5 mg/kg/day of DA. Treated animals showed no clinical changes over the 64 days, including urinalysis, haematology and serum chemistry. At the end of the study and under anesthesia, rats were exanguinated and perfused with neutral buffered formalin (NBF) or with 2% glutaraldehyde (GL): 2% paraformaldehyde (PF). Tissues were then processed for light microscopy (LM) or for electron microscopy (EM). LM results were unremarkable, including the area postrema, hippocampus and retina which are considered target sites for DA toxicity. Glial fibrillary acid protein (GFAP) and glutamate receptor immunohistochemistry (GluR1-4) did not show visually detectable difference between treated and control rats. EM of the CA3 field of the hippocampus from the high dose (5mg/kg/day) group revealed neuronal damage: cytoplasmic vacuolation, neuronal shrinkage, dilatation of dendritic and astrocytic processes and formation of electron dense profiles. This dose was equivalent to the estimated maximum human dose during the Canadian ASP incident in 1987 and is seven times less than that required to cause overt clinical signs in the rat. No significant changes were observed at the dose of 0.1 mg/kg, estimated as the dose resulting from the consumption of one 250 mg portion of mussel meat containing the present limit for DA of 20ug/g of shellfish meat.

803.12

SERUM CLEARANCE OF DOMOIC ACID IS UNALTERED FOLLOWING MULTIPLE EXPOSURES IN MICE.

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Domoic acid (DA), a tricarboxylic amino acid, is a rigid analogue of the neurotransmitter L-glutamate and has been demonstrated to be an environmental neurotoxin to humans. Previous studies in rodents and primates indicate that DA is cleared from serum within four hours after a single dose. In the present study, we have determined the concentration of DA in serum after single or multiple dose exposure and correlated this with stereotypic neurological effects in mice. Mice were intraperitoneally administered 2.0 mg/kg DA either in multiple doses (every other day i.p. for 4 times in 7 days) or a single dose on the same day of the last multiple exposure dose. DA levels were monitored as serum concentration and measured at 60 min and 120 min using the DA radioreceptor assay. Serum DA levels did not differ at 60 min in single (0.59 ± 0.04 µg/ ml) and multiple (0.50 ± 0.05 µg/ml n= 8) exposures, or at 120 min (single 0.124 ± 0.005 µg/ml, multiple 0.123 ± 0.004 µg/ml, respectively n= 7). The onset of stereotypic neurological effects in the form of scratching was also similar in each group (20.2 ± 0.5 min & 22.4 ± 1.1 min, respectively) although the duration of scratching lasted longer in mice with a single dose (30-45 min) than those with multiple exposure (20-35 min). Convulsive behavior, evident with a single 2.0 mg/kg dose, was not observed after the second dose in the multiple exposure group. This study indicates that multiple exposures of DA to mice does not alter DA clearance from the serum and does not appear to lead to a more neurotoxic response.

804.1

DISTRIBUTION OF $GABA_A$ RECEPTOR α1-SUBUNIT POLYPEPTIDES IN THE GUINEA PIG HIPPOCAMPUS. E.M. Barnes, Jr.*, M.E. Díaz, L.V. Colom, J.D. Miranda, B.J. Baumgartner, and M.H.J. Tehrani, Depts. of Neurology and Biochemistry and Div. of Neuroscience, Baylor Col. of Med., Houston, TX 77030.

In order to conduct a histochemical examination of $GABA_A$ receptors in various brain tissues, we have prepared polyclonal antibodies against a selective intracellular loop region of the α1 subunit. The chick $GABA_A$ receptor α1(331-381) subunit sequence was expressed as a fusion protein containing a hexahistidyl leader peptide, purified by Ni^{2+}-affinity chromatography, and used for rabbit immunizations. The corresponding α1-subunit antiserum immunoprecipitated 66% of ^{3}H-flunitrazepam binding to extracts from guinea pig hippocampus and reacted with a single 50-kDa polypeptide on Western blots. The strong cross-species reactivity was expected from the 88% sequence identity in the α1(331-381) region between chicken and rodents. The pattern of immunoreactivity on 50 μm sections of guinea pig hippocampus was examined using rhodamine-labeled anti-rabbit IgG and confocal laser microscopy. Immunofluorescent labeling of $GABA_A$ receptor α1 subunits was observed on pyramidal cells and putative interneurons in the CA1 and CA3 regions. The α1 subunit was located in the soma and apical dendrites of pyramidal cells. This distribution was similar for the both the CA1 and CA3 layers. Putative interneurons of the stratum oriens, pyramidale, and radiatum also showed immunoreactivity of α1 subunits.

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804.2

ANTIBODIES TO THE β-SUBUNITS OF $GABA_A$ RECEPTORS. Ming Li* and Angel L. De Blas. Div. Mol. Biol. and Biochem., School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64110-2499.

Antisera to the β_1, β_2 and β_3 subunits of rat $GABA_A$ receptor have been made by immunizing rabbits with either synthetic peptides or bacterial fusion proteins. The peptides corresponded to amino acid residues 382-393, 382-393 and 380-391 of the rat $ß_1$, $ß_2$, and β_3 subunits respectively. The large intracellular loop (IL) located between the putative transmembrane domains M3 and M4 of the rat β_1, β_2 or β_3 were expressed as staphylococcal protein A fusion proteins. The antisera specifically reacted with the corresponding peptide or IL fusion protein in ELISA. The antibodies immunoprecipitated the solubilized $GABA_A$/Benzodiazepine receptors from rat brain. Quantitative immunoprecipitation showed that in the rat cerebral cortex β_2 is the most abundant of the ß subunits whereas in the hippocampus the most abundant is $ß_3$. Immunoprecipitation studies in several brain regions showed a good correlation between the relative abundance of $ß_1$, $ß_2$, and β_3 peptides and mRNA expression. The antibodies were affinity-purified on immobilized peptide or IL protein. The latter was purified after controlled protease cleavage of the fusion protein. The affinity-purified antibodies are being used for receptor characterization by immunoblotting and immunocytochemistry.

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804.3

DISTRIBUTION OF $GABA_A$/BENZODIAZEPINE-RECEPTORS IN THE BRAIN OF GOLDFISH (*CARASSIUS AURATUS*) AND SALMON (*SALMO SALAR*). M. Anzelius*, B. I. Holmqvist and P. Ekström. Dept. of Zoology, University of Lund, Sweden.

The antibody bd-17 was used for immunocytochemical detection of $GABA_A$/benzodiazepine receptor $ß_2/ß_3$-subunits (bd-17ir) in the brain of the goldfish. The relative density and regional distribution of bd-17ir were analysed by computerized image analysis and were compared with our previous results from the salmon. In the goldfish bd-17ir is mostly found as a diffuse labelling of circumscribed areas corresponding with cytoarchitectonally defined "nuclei". Strong labelling is located in the telencephalon, pretectum, optic tectum, hypothalamus and torus semicircularis. Several nuclei of the hypothalamus and posterior tuberculum show strong labelling (e.g. the preglomerular nuclei, n. anterior tuberis, n. posterior tuberis, lobi inferiores). The stratum periventriculare, stratum griseum centrale and stratum fibrosum et griseum superficiale of the optic tectum display bd-17ir. Perikaryal labelling was observed in the torus longitudinalis and in the granular layer of different subdivisions of the cerebellum. In the brain stem the central grey is strongly immunoreactive. The bd-17ir in the goldfish brain is in general similar to that found in the salmon, but in the goldfish labelling generally correlates better with cytoarchitectonic entities, particularly in the hypothalamus and posterior tuberculum. The labelling in the central grey is not present in the salmon brain.

804.4

IMMUNOCYTOCHEMISTRY OF A NOVEL GABA RECEPTOR SUBUNIT

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Following our recent cloning of a novel γ-aminobutyric acid (GABA) receptor subunit gene *Resistance to dieldrin* or *Rdl* from the cyclodiene resistance locus in *Drosophila melanogaster*, we were interested in defining its pattern of expression during development. Here we report the raising of an anti-*Rdl* polyclonal antibody that recognizes a single protein of the expected 65 kDa size in immunoblots of *Drosophila* head homogenates. *In situ* hybridization using *Rdl* cDNA probes and the anti-*Rdl* antibody shows that *Rdl* message and protein are expressed globally in the developing central nervous system (CNS) of 15-17 hr embryos. No message can be observed on or before 12-13 hr. Interestingly, despite the use of GABA in both the peripheral and CNS of insects, *Rdl* GABA receptor subunits appear to be confined to the CNS. Detailed immunocytochemistry of *Drosophila* brain sections showed particularly strong anti-*Rdl* antibody staining in the optic lobes, ellipsoid body, fan shaped body, ventrolateral protocerebrum and the glomeruli of the antennal lobes. Results are compared with the distribution of staining observed in the insect CNS with antibodies against GABA itself, synaptotagmin (a synaptic vesicle protein) and a second *Drosophila* subunit which appears to be a homolog of the vertebrate $GABA_A$ receptor β subclass.p

804.5

FUNCTIONAL COMPARTMENTALIZATION OF $GABA_A$-RECEPTOR SUBTYPES IN THE RAT SPINAL CORD. H. Mohler*, J.M. Fritschy, and S. Bohlhalter. Institute of Pharmacology, University of Zürich, CH-8057 Zürich, Switzerland.

To assess the significance of the structural heterogeneity of $GABA_A$-receptors, we analyzed immunohistochemically the subunit composition and cellular localization of $GABA_A$-receptor subtypes in the rat spinal cord. The regional distribution of the subunits α1, α2, α3, α5, β2,3, and γ2 was investigated with subunit-specific antibodies, and their co-localization within individual neurons visualized by double-immunofluorescence staining. The results reveal a widespread and almost ubiquitous expression of the subunits α3, β2,3, and γ2 in the spinal cord, suggesting that most receptors comprise the subunit triplet αx/βx/γ2, which forms functional receptors amenable to benzodiazepine modulation. An "atypical" $GABA_A$-receptor subtype, containing the subunits α2/γ2 only, was detected in preganglionic and somatic motoneurons. Further heterogeneity arose from the differential, lamina-specific distribution of the subunits α1 and α5 in layers III-VIII. Thus, in lamina III, most neurons contained either the subunit α1 and/or α5, along with β2,3 and γ2. In laminae IV-V, the most frequent α-subunit was α1, whereas in laminae VII-VIII, the subunit α5 predominated (along with the subunits α3/β2,3/γ2). However, the α3- and α5-subunits were located to distinct cells. Hot-spots of immunoreactivity, suggestive of a postsynaptic localization of $GABA_A$-receptors, were seen frequently on neuronal somatic and dendritic membranes. In addition, an intense and diffuse staining was observed for the subunits α2/α3/β2,3/γ2 in laminae I-III, localized presumably on primary afferent terminals. These results demonstrate the existence of different $GABA_A$-receptor subtypes in distinct compartments of the spinal cord, suggesting that $GABA_A$-receptor heterogeneity may be of relevance for the modulation of sensory inputs, nociception, and motor control at segmental levels.

804.6

DIFFERENTIAL DISTRIBUTION OF $GABA_A$ RECEPTOR SUBUNITS IN THE RAT INFRALIMBIC CORTEX: RELEVANCE TO NOVEL ANTIPSYCHOTIC DRUG TREATMENT. E.Dunn*, J.M.Fritschy[1], D.B.Carter and K.M. Merchant, CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001 and [1]Institute of Pharmacology, Univ. of Zurich.

The prefrontal cortex has been implicated in manifestation of negative symptoms and cognitive deficits in schizophrenic patients. Several studies of postmortem human tissue indicate that there is a loss of GABAergic interneurons and an increase in $GABA_A$ receptor binding in the prefrontal cortical regions of schizophrenics. These data suggest that disturbances in GABAergic neurotransmission in limbic cortex of schizophrenic patients may contribute to the pathophysiology of schizophrenia, and that specific GABAergic agents may be therapeutically useful. To evaluate this possibility, we have performed a detailed analysis of distribution of $GABA_A$ receptor subunits in the rat medial prefrontal cortex (infralimbic, IL, region). Mapping of α_1, α_2, α_3, α_5 and β_{2+3} subunits was carried out by immunocytochemistry with previously characterized, subunit-specific antibodies. The α_1 and β_{2+3} subunit antibodies immunostained all layers (I, II, III, V, and VI) of the IL cortex, with layers II and III displaying what appeared to be small interneurons. In contrast, α_2, α_3 and α_5-like immunoreactivity was restricted to a single layer of the IL region; layer I displayed labelling for α_2 and layer VI for α_3 and α_5 subunits. Both α_3 and α_5 antibodies immunostained large cell bodies, resembling pyramidal cells, which appeared to be restricted to layer VIb for the α_5 subunit. Such studies may help design pharmacological agents with subunit selectivity to modulate GABA neurotransmission in a regionally-selective manner.

804.7

COMPARISON OF DISTRIBUTION PATTERNS OF GABA AND GABA$_A$ RECEPTORS IN THE RAT NUCLEUS TRACTUS SOLITARIUS.

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GABA, an inhibitory neurotransmitter, plays an important role in the nucleus tractus solitarius (NTS). The nucleus is associated with taste sensation, general visceral input and regulation of the autonomic nervous system. Previous immunohistochemical studies have demonstrated that GABA-positive terminals and neuronal somata are richly distributed in the NTS.

In this study, we examined the immunohistochemical localization of GABA$_A$ receptors in the rat NTS using a monoclonal antibody against the b-subunit. The pattern of distribution was compared with that of presynaptic GABA terminals. Immunoreactivity for GABA$_A$ receptors was confined to the surface of neuronal somata and processes. These positive structures were distributed mainly in the rostral part of the NTS, where GABA terminals were also abundant. In the intermediate and caudal parts of the NTS, however, positive staining for GABA$_A$ receptors was located in the ventrolateral part of the NTS, particularly in the ventrolateral subnucleus. In these regions, GABA-positive terminals were rather sparse. In addition, few if any cells positive for GABA$_A$ receptors were seen in the medial part of the NTS, including the medial subnucleus where GABA-positive terminals were dense. The results clearly indicate that GABA neurons probably act in the rostral NTS via GABA$_A$ receptors but that their actions in the intermediate and caudal parts of the NTS appear to be mediated by receptors that are not detected by the monoclonal antibody used.

804.8

Ba^{2+}-SENSITIVITY OF MONOSYNAPTIC AND 4-AP-INDUCED GABA$_B$ IPSPs IN CA1 PYRAMIDAL CELLS OF RAT HIPPOCAMPAL SLICES.

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GABA$_B$ receptors in hippocampal pyramidal cells may be coupled to heterogeneous K^+ conductances that are differentially sensitive to Ba^{2+}. The aim of this study was to examine with intracellular recordings the effects of barium on GABA$_B$-mediated inhibitory postsynaptic potentials (IPSPs), pharmacologically isolated in the presence of GABA$_A$, NMDA and non-NMDA receptor antagonists, in conventional rat hippocampal slices. In a first series of experiments, monosynaptic GABA$_B$ IPSPs were evoked by stimulation of GABA fibers/cells in str. radiatum, lacunosum-moleculare or oriens. These IPSPs displayed the following characteristics (mean ± sem): amplitude -5.4±0.5 mV, peak latency 150±22 ms, and recovery time 386±37 ms (n=15). The E_{rev} of these IPSPs was -91.6±2.2 mV (n=12). Bath application of 1mM Ba^{2+} completely suppressed monosynaptic GABA$_B$ IPSPs to -35.9±16.0% of control amplitude (n=12; p<.001). Ba^{2+} effects were reversible upon washout (42.0±19.4% of control amplitude; n=4). In a second series of experiments, spontaneous GABA$_B$ IPSPs were induced by bath application of 100 µM 4-aminopyridine (4-AP). Spontaneous 4-AP induced GABA$_B$ IPSPs had the following properties: amplitude -8.1±0.6 mV, rise time 215±20 ms, recovery time 1.8±0.4 s (n=10). Their E_{rev} was -107.2±3.6 mV (n=7). Synchronized GABA$_B$ IPSPs were progressively abolished by bath application of Ba^{2+} (n=8). Spontaneous GABA$_B$ IPSPs recovered after washout of Ba^{2+} (64.8±5.4% of control amplitude, n=7). These results indicate that GABA$_B$ IPSPs, either evoked monosynaptically or synchronously induced in 4-AP, were mediated by K^+ conductances sensitive to Ba^{2+}. Thus, synaptically released GABA may not activate postsynaptic GABA$_B$ receptors linked to Ba^{2+}-insensitive K^+ conductances.

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804.9

KINETIC PROPERTIES OF EVOKED MONOSYNAPTIC FAST IPSCS IN NEOCORTICAL PYRAMIDAL CELLS.

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Previously, we examined the recruitment properties of fast IPSCs in neocortical layer V pyramidal cells (PCs) and found that the magnitude of fast GABAergic inhibition is absolutely limited. However, overall inhibitory strength is also determined by IPSC kinetics. For example, if IPSC time decay is prolonged as a consequence of volume transmission, an increase in total charge transfer results. We have, therefore, examined the kinetic properties of fast IPSCs using whole-cell techniques to record from layer V PCs in slices (400µm) of somatosensory cortex from young (P15-30) rats. Fast IPSCs were evoked with graded stimuli applied to layer V/VI. Cesium-gluconate electrode solutions were employed to block slow GABA$_B$-mediated IPSCs and excitatory synaptic transmission was blocked with CPP and CNQX (10µM). Isolated IPSCs were best fit with an equation describing second-order activation and single-exponential current decay. There was no apparent correlation between IPSC kinetics and stimulus intensity. When IPSCs reached maximal amplitude, further increases in stimulus intensity did not cause significant changes in either IPSC rise or decay. The consequences of these kinetic properties for limited fast inhibition and the underlying pre- and postsynaptic mechanisms will be discussed. (Supported by MH51677)

804.10

DUAL MODULATION OF GABA$_A$ RECEPTORS BY EXTERNAL H^+ IONS IN ACUTELY ISOLATED RAT HIPPOCAMPAL NEURONES

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We have studied the effect of extracellular H^+ on the GABA$_A$ receptor-mediated chloride conductance in acutely isolated pyramidal neurones from the rat hippocampus under whole-cell voltage clamp in HCO_3^--free solutions. The conductance evoked by saturating concentrations (500-1000 µM) of GABA showed a marked sensitivity to variations in external pH (pH_o) around 7.4. A rise in pH_o between 6.4 and 8.4 decreased the conductance by about two-fold per pH unit. However, when evoked by low GABA concentrations (1-10 µM) the conductance showed an equally marked increase upon an increase in pH_o. At an intermediate concentration of GABA (around 30 µM), the GABA$_A$ conductance was not affected by external H^+. The concentration-response relationship was flat between 50-100 µM GABA, consistent with the presence of at least two functionally distinct GABA$_A$ receptors with different affinities. The effect of pH_o was best described as a combination of two effects: i) a downmodulatory effect of H^+ seen as a parallel shift to the right in the concentration-response curve of the higher affinity GABA receptor, and ii) an upmodulatory effect of H^+ seen as a non-competitive potentiation of both the higher and the lower affinity receptor populations. Zn^{2+} (1-50 µM) inhibited in a concentration-dependent manner the conductance induced by saturating GABA concentrations. In addition, at pH_o 8.4 Zn^{2+} induced a parallel positive shift in the GABA concentration-response curve. In the presence of Zn^{2+} the GABA$_A$ conductance was upmodulated by H^+ at both high and low agonist concentrations. The above data implie a coexistence in these cells of two functionally distinct GABA$_A$ receptor populations with different affinities to GABA and different sensitivities to H^+ and Zn^{2+}. The high sensitivity of GABA$_A$ receptors to H^+ suggests that the efficacy of central inhibition depends on the regulation and modulation of interstitial pH in the brain.

804.11

GABAergic MODULATION OF NEURONS FROM THE HORIZONTAL LIMB OF THE DIAGONAL BAND OF BROCA (hDBB) THAT PROJECT TO THE HIPPOCAMPUS.

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Anatomical data indicate reciprocal projections between the hDBB and the hippocampus. GABA has been identified as a major transmitter in these pathways, which have been implicated in the generation and maintenance of the hippocampal theta rhythm. We have examined the actions of GABA receptor agonists on acutely, dissociated rat hDBB neurons using the whole-cell patch-clamp technique. Fluorescein-labelled latex microspheres were injected into the hippocampus to retrogradely label cells in the hDBB. Three to four days later, cells were acutely dissociated using enzymatic treatment and visualized under fluorescent microscopy to identify the labelled neurons. Current-voltage relationships of the labelled hDBB neurons were similar to those recorded from unlabelled cells. Under voltage-clamp conditions, bath applied muscimol (10µM), a GABA$_A$ receptor agonist, evoked a current that reversed at -69 mV (n=4). Baclofen (10µM), a GABA$_B$ receptor agonist, evoked no response (n=4). These results suggest that GABAergic afferents modulate the activity of the hDBB neurons projecting to the hippocampus through the GABA$_A$ receptor.

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804.12

INHIBITORY POSTSYNAPTIC POTENTIALS IN RAT SUBICULAR BURSTING NEURONS.

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Intracellular recordings from rat subicular bursting neurons (BNs, n=35) were made in an in vitro slice preparation to evaluate the inhibitory component of the response to single-shock extracellular stimuli delivered in different portions of the CA1 subfield. Stimulation of the CA1 alveus and CA1 stratum radiatum evoked a sequence of depolarizing-hyperpolarizing potentials in 17 BNs, whereas only a monophasic depolarization was observed in 16 BNs. Stimuli applied either to the CA1 stratum pyramidale or stratum lacunosum-moleculare induced in the same cells (n=2) a depolarizing potential and a depolarizing-hyperpolarizing sequence of potentials, respectively. When varying the resting-membrane potential the stimulus-induced hyperpolarization behaved as expected for an inhibitory postsynaptic potential (IPSP), had a reversal potential of -81 ± 5 mV (n=10) and was associated with an increase in membrane conductance of 25 ± 14 nS (n=5). Shunt of the action potentials induced by prolonged depolarizing current injection occurred when hyperpolarizing IPSPs were elicited by concomitant synaptic stimulation. Bath application of the GABA$_A$ receptor antagonist BMI (10µM, n=4) reduced both the IPSP amplitude and the associated increase in membrane conductance. These findings suggest that only a subset of subicular BNs respond with a hyperpolarizing IPSP when activated by the stimulation of different portions of the CA1 subfield. They also indicate that this hyperpolarizing IPSP is mainly due to the postsynaptic activation of GABA$_A$ receptors located on BNs. Supported by Savoy Foundation and MRC of Canada.

804.13

A BICUCULLINE- AND SACLOFEN-RESISTANT GABA CURRENT IN SUBSTANTIA GELATINOSA OF THE RAT SPINAI CORD *IN VITRO*. M. Yoshimura*, H. Baba, Y. Yajiri and H. Higashi. Dept. Physiol., Kurume Univ. Sch. Med. Kurume 830, Japan.

Stimulation of Aδ afferent fibers evoked a GABAergic IPSP via the $GABA_A$ receptor. In many SG neurons, however, the GABA-induced response was in part resistant to bicuculline and saclofen. To study the property of the GABA response, blind patch clamp recordings were made from SG neurons in the transverse spinal cord slices of the adult rat. Bath applied GABA (0.1 - 1 mM) produced an outward current which consisted of an initial peak and a slowly decaying plateau. The initial peak current was depressed by bicuculline (10 - 100 μM), while the plateau current was hardly affected. $GABA_B$ receptor antagonist, phaclofen (500 μM) and saclofen (500 μM) also had no significant effect on the plateau current. Picrotoxin (60 - 100 μM) depressed the GABA current by more than 60 %. Both the initial peak and plateau currents were reversed in polarity near the Cl^- equilibrium potential. It appears, therefore that the plateau current has a similar property to the $GABA_C$ current reported recently in visual system. The $GABA_C$ receptor analog, *trans*-4-aminocrotonic acid (CACA, 0.5 - 1 mM), in fact, produced in SG neurons a response similar to the GABA response The CACA-induced current was, however, completely abolished by bicuculline (20 μM). These observations provide an idea that SG neurons may express a novel GABA receptor which has a different pharmacological property from the $GABA_C$ receptor and that this receptor may be involved in modulation of nociceptive transmission in the spinal dorsal horn.

804.14

MUSCIMOL INDUCES TWO DISTINCT ELECTROPHYSIOLOGICAL RESPONSES IN RAT SUPRACHIASMATIC NUCLEUS NEURONS *IN VITRO*. J.A. Zidichouski*, S.B Kombian and Q.J. Pittman Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada.

The Suprachiasmatic Nucleus (SCN) is implicated as an important hypothalamic site involved in the synchronization of physiological functions that are circadian by nature. Sleep/wake cycles, body temperature, cardiovascular and digestive functions are influenced by cues obtained from the environment. Circadian rhythms are affected by light, the pineal hormone melatonin, and by a variety of pharmacological agents. For example, benzodiazapines cause a significant phase advance in wheel running activity in rodents. GABA immunoreactivity is evident throughout the SCN and GABAergic interneurons are thought to mediate inhibitory postsynaptic potentials recorded within this nucleus. The present study, therefore, examined the electrophysiological effects of exogenous application of the $GABA_A$ receptor agonist muscimol in an in vitro SCN slice preparation (400 μm coronal slices). The whole-cell nystatin patch clamp technique was used and recordings were made from 21 cells located in the dorsal aspect of the SCN. Bath application of muscimol (5-100μM) produced two distinct electrophysiological responses. In 13 of 21 (62%) SCN neurons, a membrane depolarization or inward current was observed. This "atypical" response reversed at -57.7 ±2.1 mV (n=6) and was accompanied by a marked decrease in membrane resistance (26.4% of control ±9.7, n=7). A typical muscimol-induced hyperpolarization or outward current that reversed at -73.8 ±1.1mV (n=6) was observed in the 8 remaining SCN neurons. The mean change in input resistance was 72.3% of control (±7.5% n=3). Both responses were, however, attenuated by the $GABA_A$ channel blocker picrotoxin (25-100μM). Supported by MRC Canada (QP & SK) & Ciba-Geigy Canada Ltd. (JZ)

804.15

PENTOBARBITAL MODULATION OF GABA-A RECEPTOR KINETICS USING ULTRAFAST ACTIVATION IN EXCISED PATCHES OF MOUSE CORTICAL NEURONS. J.J. TINGEY, A.M. LAVOIE, J.R. BARINGER* & R.E. TWYMAN. Programs in Neuroscience, Human Molecular Biology & Genetics, Depts of Neurology & Pharmacology, Univ. of Utah, Salt Lake City, UT

Steady state applications of pentobarbital (PB) have previously been shown to increase GABA-A mediated chloride current by increasing single channel open duration without changing open frequency [Macdonald et al., J. Physiol., 1989]. An ultrafast ligand exchange system (100μs exchange time) was used to examine the effects of PB on GABA binding and gating kinetics (channel activation and relaxation). Outside-out patches from cultured fetal mouse cortex contained >10 channels and were voltage clamped at -75mV in symmetrical chloride solutions at room temperature. GABA (10μM-10mM) alone or in the presence of PB (50μM) was applied to patches using either a series of repeated brief pulses (800μs) or single step applications (50ms-2s). Chloride currents were sampled at 20-100kHz and filtered at 2-10kHz. Current onset (10-90% rise time) and decay time constants (τ) were analyzed individually and as patch ensemble averages.

Pre-application of PB was necessary to see an alteration of kinetics, suggesting that PB binds the receptor more slowly than GABA. As we previously reported for diazepam, PB did not alter channel maximal opening rates. Unlike diazepam, PB prolonged current relaxation indicating that PB increases time spent in one or more bursting states, similar to that seen for steady state single channel recordings.

NEUROPEPTIDE LOCALIZATION: CNS REGULATION

805.1

DISTRIBUTION OF THE NEUROPEPTIDE Y Y2 RECEPTOR mRNA IN RAT BRAIN. E.L. Gustafson*, M.M. Durkin, K.E. Smith, C. Gerald, Y. She, A.I. Illy, R.W. Weinshank and T.A. Branchek. Synaptic Pharmaceutical Corporation, Paramus, NJ 07652.

The expression cloning of the human NPY Y2 receptor cDNA and the subsequent cloning of the rat homologue (C. Gerald et al., K.E. Smith et al., Soc. Neurosci. Abstr. 1995) have made it possible to localize the mRNA encoding this NPY receptor subtype in rat tissues. We have carried out *in situ* hybridization studies using [^{35}S]dATP-labelled oligonucleotide probes to the rat Y2 receptor mRNA to determine the distribution of Y2 mRNA in the rat brain and peripheral tissues.

Probe specificity was confirmed by testing the radiolabelled oligonucleotides in transfected cells. In rat forebrain, Y2 mRNA is most abundant in the CA3 region of the hippocampus, the medial nucleus of the amygdala, and in the arcuate nucleus and tuber cinereum of the hypothalamus. Hybridization signals are also observed over the olfactory tubercle, the lateral septal area, the basomedial and cortical nuclei of the amygdala, the dorsomedial and ventromedial hypothalamus, the dorsal and ventral premammillary nuclei, the piriform cortex, and the centromedial and paraventricular nuclei of the thalamus. Caudally, hybridization signals for the Y2 mRNA are restricted to the dorsal and caudal linear raphe, the pontine nucleus, and the posterior dorsal tegmental nucleus. In the spinal cord, labelling is observed over scattered large neurons in lamina 9. A subpopulation of large neurons in the dorsal root ganglia also exhibit a low hybridization signal for the Y2 mRNA.

The present results indicate that the mRNA encoding the Y2 receptor is discretely localized in the rat brain. In some areas, colocalization of this novel receptor with NPY itself appears likely, particularly in the arcuate nucleus of the hypothalamus. The distribution of this NPY receptor subtype mRNA suggests involvement in multiple physiological roles.

805.2

ASSOCIATIONS BETWEEN NEUROPEPTIDE Y NEURONAL ELEMENTS AND MICROVESSELS IN RAT AND HUMAN CEREBRAL CORTEX. R. Abounader* & E. Hamel, Montreal Neurological Institute, McGill Univ, Montréal, QC, Canada H3A 2B4

Neuropeptide Y (NPY) constricts cerebral intracortical arterioles (Dacey *et al.* 1988 *J. Cereb Blood Flow Metab* 8: 254) and NPY-containing nerve terminals have been incidentally observed in apposition with such intracortical microvessels (Aoki & Pickel 1989 *J Neurosci* 9: 4333). To assess the morphological basis for a putative role of NPY in the regulation of microvascular functions, we performed a quantitative study of the associations of NPY neuronal elements with microvessels in human and rat cerebral cortex, respectively fixed by immersion after surgical removal and by intra-aortic perfusion. Brain sections were immunostained for NPY and analysed using light microscopy. On 20 μm-thick sections, the overall association of NPY neurons with local intracortical microvessels was evaluated. On semithin (2 μm-thick) sections, the total number of NPY neuronal elements, blood vessels and perivascular NPY punctate structures were counted on photomicrographs. Only neuronal elements which directly touched a vessel wall were considered to be perivascular. In the 20 μm-thick sections of rat cerebral cortex, many NPY-containing neurons showed associations with intraparenchymal vessels, these were more frequent between NPY processes and vessel walls than between cell bodies and vessels. On semithin sections in the rat, 3.6% of all NPY punctate structures (n=4709) were perivascular. Also, of all vessels counted (n=476), 22.5% were contacted by at least one NPY element with 7.6% of the vessels having 2 or more NPY processes. In man, an extremely dense network of NPY-containing neuronal elements with very few cell bodies was observed in both thick and semithin sections. Due to the inability to visualize all intracortical vessels and to the very high density of the NPY-innervation in the human cortex, a quantitative analysis was not performed. However, many of the processes, most likely nerve terminals, were apposed to a vessel wall in semithin sections. These morphological data may indicate that cortical microvascular functions are regulated by NPY neuronal mechanisms. Further studies at the ultrastructural level would help define these NPY neurovascular relationships. Supported by Dr Karl Thomae GmbH/Bio-Mega and the MRC of Canada through a University-Industy grant.

805.3

PRIMARY HIPPOCAMPAL NEURONAL CULTURES CO-EXPRESS NPY-LIKE IMMUNOREACTIVITY AND NPY Y1 RECEPTORS: A POSSIBLE SELF-AUTOREGULATORY MECHANISM? J.-A. St-Pierre*, Y. Dumont, and R. Quirion. Douglas Hosp. Res. Ctr. and Dept. Neurol. and Neurosurg., and Psychiatry, McGill University, Montréal, Québec, Canada, H4H 1R3.

Neuropeptide Y (NPY) and its homologues induce complex effects in the hippocampal formation that include the modulation of glutamatergic neurotransmission (Colmers and Bleakman *TINS* **17**:373,1994) and sigma receptor systems (Monnet et al. *JPET* **263**:1219, 1992; Bouchard et al. *J. Neurosci.* **13**:3926,1993). At least two classes of NPY receptors, Y1 and Y2 , mediate these functional effects as both sub-types are expressed in the rat hippocampus, albeit each according to a unique anatomical profile (Dumont et al., *J.Neurosci.***13**:73, 1993). In order to investigate further the respective role of the Y1 and Y2 receptors at the cellular and molecular levels, a rat embryonic primary hippocampal neuronal cell culture model was developed and shown to be uniquely enriched with Y1/[^{125}I][Leu^{31},Pro^{34}]PYY binding sites (St-Pierre et al. *Soc. Neurosci. Abst* **20**:85, 1994). The aim of the present study was to establish the phenotype(s) of Y1 receptors-bearing hippocampal neurons using a combined immunocytochemical/emulsion receptor autoradiographic method and focusing first on the possible existence of NPY-immunoreactive neurons in the cultures. Dissociated hippocampal cells from embryonic days 18-19 rats were grown for 20 days on cover-slips. Emulsion receptor autoradiography was then performed using the selective Y1 radioligand, [^{125}I][Leu^{31},Pro^{34}]PYY (35pM), followed by NPY immunocytochemistry (ABC method, Vector) with a highly specific NPY antibody (generously provided by G. Pelletier, Laval Univ.). Most interestingly, a significant proportion of bipolar and pyramidal-like neurons were clearly NPY-immunoreactive and expressed the Y1 receptor sub-type. These results suggest the possible existence, via Y1 receptors, of an autoregulatory mechanism governing NPYergic neurotransmission in the rat hippocampal formation. (Supported by the MRCC and the FCAR).

805.4

PREPRO-NEUROPEPTIDE Y mRNA EXPRESSION AND RECEPTOR AUTORADIOGRAPHY IN BRAIN FROM HYPERTENSIVE AND NORMOTENSIVE RATS K. J. McLean, B. Jarrott and A. J. Lawrence*. Dept. of Pharmacology, Monash Univ., Clayton, Victoria, 3168, Australia.

Neurons containing neuropeptide Y (NPY) may participate in central cardiovascular control by tonically suppressing the baroreceptor reflex pathway, possibly by influencing barosensitive neurons within the nucleus tractus solitarius (NTS) (1). The present study has employed both in situ hybridization histochemistry and receptor autoradiography, to visualise the expression of preproNPY mRNA in the forebrain and to determine the NPY receptor subtype/s in the brainstem, respectively. Specific hybridization signals to a preproNPY antisense oligonucleotide probe were visualized in the cortex, dentate gyrus, hippocampus and reticular thalamus from age-matched spontaneously hypertensive rats (SHR) and normotensive Don Ryu rats (DRY) and Wistar-Kyoto rats (WKY). Differences in the expression of preproNPY mRNA were only observed in the hypothalamic arcuate nucleus, where densitometric analysis of images revealed values of 4.21 ± 0.39 (SHR; n=7), 1.81 ± 0.53 (DRY; n=8) and 1.42 ± 0.15 dpm/mm^2 (WKY; n=8). Autoradiography using ^{125}I-Bolton Hunter-NPY (BH-NPY, 15 pM) demonstrated NPY binding sites in the dorsal vagal complex and inferior olive. NPY (1 µM) and peptide YY (1 µM), but not [Leu^{31}, Pro^{34}] NPY (10 - 100 nM), fully inhibited the binding of ^{125}I-BH-NPY. These results indicate that NPY receptors of the Y_2 subtype predominate in the dorsal vagal complex. While all three rat strains appear to have the same receptor subtype in the brainstem, the relevance of the differential NPY gene expression in the arcuate nucleus regarding central cardiovascular control mechanisms and /or the pathogenesis of hypertension remains to be elucidated.

1. Shih, C.-D. et al., (1992) Neurosci. Lett. 148, 169-172.

805.5

[^{3}H]BIBP3226, A NEWLY DEVELOPED NON-PEPTIDE NEUROPEPTIDE Y Y_1 RECEPTOR ANTAGONIST RADIOLIGAND: BINDING CHARACTERISTICS AND QUANTITATIVE AUTORADIOGRAPHY.

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Rudolf et al. (Eur. J. Pharmacol., 271: R11-R13, 1994) recently reported the development of a highly selective non-peptide neuropeptide Y (NPY) Y_1 receptor antagonist, devoid of activity on the Y_2 and Y_3 (Jacques et al., Eur. J. Pharmacol. in press) receptor subtypes. This suggests that BIBP3226, in a radiolabelled form, could prove most useful as the first antagonist radioligand to investigate Y_1 receptor binding parameters. Entzeroth et al. (Eur. J. Pharmacol., in press) have just developed [^{3}H]BIBP3226. This new radioligand was studied here using both rat brain membrane binding assays and receptor autoradiography according to protocols described earlier (Dumont et al., J. Neurosci., 13: 73-86, 1993). Unlabelled BIBP3226 competed with high affinity for rat brain Y_1 (Ki of 1 nM) but not Y_2 (> 1 µM) binding sites. Autoradiograms revealed that 100 nM BIBP3226 competed for at least 75 to 90% of 30 pM [^{125}I][Leu^{31},Pro^{34}]PYY/Y_1 agonist binding sites in most brain regions including in the superficial layers of the cortex, various thalamic nuclei, the dentate gyrus of the hippocampus and the cerebellum. In contrast, few areas such as the glomerular layer of the olfactory bulb, lateral septum, nucleus tractus solitarius (NTS) and area postrema are rather resistant (<30%) to BIBP3226. The direct use of [^{3}H]BIBP3226 (5 nM) as radioligand confirmed and extended these findings with high specific labelling concentrated in cortical areas and in the thalamus for example, but not in the NTS and the area postrema. Taken together, these results may suggest the possible existence of Y_1 receptor subtypes, one being highly sensitive to BIBP3226. Supported by an MRCC Industry/University Grant jointly with Thomae GmbH/Bio-Mega.

805.6

NEUROPEPTIDE Y Y_1 AND Y_2 RECEPTOR BINDING SITES IN THE RAT AND HUMAN SPINAL CORD: EFFECT OF SURGICAL MANIPULATIONS AND AMYOTROPHIC LATERAL SCLEROSIS (ALS). D. Jacques[1], Y. Dumont[1], S. Kar[1], C. Krieger[2], A. Fournier[3], S. St-Pierre[3] and R. Quirion[1]. [1]Douglas Hospital Res, Ctr, Dept. Psychiatry, McGill University, Montreal, Canada, H4H 1R3; [2]University of British Colombia, University Hospital, Vancouver, Canada; [3]INRS-Santé, 245 Boul Hymus, Pointe-Claire, Canada, H9R 1G6.

Autoradiographic studies have shown that following sciatic nerve sectioning, [^{125}I]BH-NPY binding decreased while [^{125}I]PYY labelling increased in the superficial laminae of the ipsilateral dorsal horn (Kar and Quirion, Brain Res.,574:333, 1992; Zhang et al., Eur. J. Neurosci., 7:367, 1995). In order to determine the neuropeptide Y (NPY) receptor subtypes involved, the respective distribution of the Y_1 and Y_2 receptors was investigated in the rat and human spinal cord using recently developed selective radioligands (Dumont et al., J. Pharmacol. Expt.Ther. 272: 673, 1995). Another series of experiments were carried out to determine whether Y_1 and Y_2 receptor binding sites are differentially altered following sciatic nerve lesion and in ALS spinal cord. Autoradiographic studies revealed that high levels of both Y_1/[^{125}I][Leu^{31},Pro^{34}]PYY and Y_2/[^{125}I]PYY$_{3\text{-}36}$ binding sites are present in laminae I and II of the dorsal horn of the adult rat spinal cord. Deeper laminae and the ventral horn are more heavily labelled with Y_2 ligand. Following unilateral sciatic nerve section (14 days), specific Y_1 binding decreased while Y_2 labelling increased in the superficial laminae of the ipsilateral dorsal horn. In contrast to the rat spinal cord, Y_1/[^{125}I][Leu^{31},Pro^{34}]PYY and Y_2/[^{125}I]PYY$_{3\text{-}36}$ binding sites are rather similarly distributed in the normal human spinal cord with labelling particularly concentrated in laminae I and II, and the ventral horn expressing low but still significant levels of binding sites. No significant alterations were noted in the distributional profile and/or density of Y_1 or Y_2 receptor subtypes in the ALS cord. Taken together, these results suggest that both the Y_1 and Y_2 receptor subtypes are expressed in the rat and human spinal cord, and are differentially regulated following sciatic nerve lesioning. Supported by the MRCC and an Industry-University grant.

805.7

DISTRIBUTION OF [^{3}H]-NEUROTENSIN RECEPTORS IN HUMAN BRAIN. Steven R. Daviss*, Carol A. Tamminga, and Robert A. Lahti. Maryland Psychiatric Research Center, University of Maryland School of Medicine, P.O. Box 21247, Baltimore, MD 21228.

The distribution of receptors for neurotensin in human brain are distributed primarily in so-called limbic areas, with a distribution that appears to be distinct from that of dopamine receptors. We have examined dopamine receptor subtype distributions within human brain, and would like to compare these distributions with that of neurotensin receptors in brain tissue from normal controls. We present here autoradiographic data using [^{3}H]-neurotensin (^{3}H-NT) to locate NT receptors in normal human brain. Two coronal hemi-blocks were cut at the level of the head of the caudate and at the level of the hippocampus from frozen human brain tissue from three individuals with no known psychiatric or neurologic illnesses. Each hemi-block was divided into 3 smaller blocks, and these were sectioned at 20µm on a cryostat, thaw mounted, incubated in ^{3}H-NT, and developed on tritium-sensitive film. Digitized images were used to estimate specific binding across blocks and cases. Areas of intense binding include anterior cingulate cortex (ACC), insular cortex, and entorhinal cortex (ERC), with moderate binding in caudate, amygdala, subiculum, and prefrontal cortex (PFC). Binding within ACC and PFC superficial cortical layers was about twice that in deep layers. Discrete areas of intense binding within the superficial layers of ERC were consistent with localization to the layer II cellular islands. These data are consistent with previous reports of distribution of [^{125}I]-NT receptors in human brain tissue.

805.8

NEUROTENSIN RECEPTOR mRNA LOCALIZATION IN HUMAN MIDBRAIN, BASAL GANGLIA, CINGULATE CORTEX AND HIPPOCAMPAL FORMATION BY IN SITU HYBRIDIZATION. S.S. Wolf[1]*, S.E. Bachus[1], B.L.Kinkead[2], M.J. Owens[2], C.B. Nemeroff[2], J.E. Kleinman[1], T.M. Hyde[1]. [1]NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032 and [2]Dept. of Psychiatry and Behavioral Sciences, Emory University School of Medicine, Atlanta, GA 30322

Neurotensin (NT) is a putative CNS neurotransmitter which may be implicated in certain neuropsychiatric disorders. High concentrations of NT receptors are present in specific brains regions, including the substantia nigra (SN), ventral tegmental area (VTA), entorhinal and cingulate cortices, with moderate concentrations in the basal ganglia. Recently, human midbrain and striatal NT receptor mRNA was localized by *in situ* hybridization (Yamada et al., *Neuroscience* 64: 405-417, 1995). We have corroborated these findings and further examined select limbic regions in which NT receptors have previously been reported to be concentrated. Utilizing a ^{35}S-labelled antisense riboprobe, we examined several rostro-caudal levels of 8 normal human midbrains. High levels of NT receptor mRNA signal were detected in the substantia nigra pars compacta and in the paranigral subnucleus of the VTA. In contrast, faint signal was detected in other subnuclei of the VTA and other mesencephalic structures. No significant signal was observed in striatum. In the hippocampal formation, moderate signal was seen in the dentate gyrus, with light labeling of layer II of the entorhinal cortex. Pregenual cingulate cortex revealed only faint signal in a homogenous pattern. The association of high NT receptor mRNA signal with dense NT receptor concentrations in some regions, such as SN, is consistent with a perikaryal localization of NT binding sites. In contrast, the significant mismatch of moderate or high receptor density in striatum, cingulate and entorhinal cortices, with faint, or absent, mRNA signal suggests that these regions may represent terminal NT binding sites.

805.9

COEXISTENCE OF VASOACTIVE INTESTINAL PEPTIDE mRNA AND NEUROTENSIN RECEPTOR mRNA IN MANY NEURONS OF THE SUPRACHIASMATIC NUCLEUS IN THE FEMALE RAT.

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Vasoactive intestinal peptide (VIP)-synthesizing neurons in the suprachiasmatic nucleus (SCN) are implicated in the regulation of gonadotropin-releasing hormone (GnRH) secretion. Recently, it has been suggested that neurotensin (NT) binding sites are closely associated with VIP-containing neurons in the SCN (Francois-Bellan et al., *Synapse* 10:282, 1992). The goal of the present study was to investigate whether, and to what extent, VIP neurons in the SCN express mRNA encoding the high-affinity NT receptor (NTR). Coronal sections were prepared from the brains of adult female rats (n = 8), and double-label *in situ* hybridization histochemistry with digoxigenin-labeled and radiolabeled (^{35}S or ^{33}P) cRNA probes was used for simultaneous detection of VIP mRNA and NTR mRNA in individual cells. As expected, both mRNAs were detected primarily in the ventral SCN, where the regional distribution of VIP mRNA labeling coincided with that of NTR mRNA labeling at all rostrocaudal levels. Although single-labeled cells of either type were observed, the vast majority of VIP mRNA-labeled cells also displayed NTR mRNA labeling. Our results provide definitive evidence that neurons capable of synthesizing VIP are by far the predominant cell type in the SCN that expresses NTR mRNA. Moreover, these results suggest that VIP-synthesizing neurons are the major cell type in the SCN in which NTR levels are reduced by estrogen, as shown in previous studies. We speculate that NT-mediated hormonal input and photic/circadian inputs are integrated in the SCN by certain VIP-synthesizing neurons which directly regulate the activity of GnRH neurosecretory cells. Supported by NIH Grant DK 29876.

805.10

DISTRIBUTION AND SEASONAL VARIATION OF VIP-LIKE PEPTIDES IN THE NERVOUS SYSTEM OF *HELIX POMATIA*.

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The distribution of neuropeptides immunologically related to vasoactive intestinal peptide (VIP) and its precursor peptide preproVIP(111-122), was studied in the central and peripheral nervous system of the snail, *Helix pomatia*, by use of immunocytochemical methods. VIP and preproVIP-related immunoreactivity were present in somata and nerve fibres in subpopulations of neurons in all central ganglia. Hibernating snails contained on average a total of 670 VIP- and 763 prepro-VIP-immunoreactive neurons. The number of immunoreactive cells was substantially reduced by more than 50% in active snails during summer with an average of 289 VIP- and 356 prepro-VIP-immunoreactive neurons. Antiserum against VIP labelled nerve fibres next to blood vessels and smooth muscle cells, whereas prepro-VIP-like material was localized in nerve fibres and endocrine-like cells. VIP-immunoreactive material was also found in accessory ganglia of small and large tentacles, ganglia of the lips, the sensory epithelium of the tentacles, free nerve endings between skin epithelial cells, neuronal cells in the retina and in the sensory epithelium of statocysts.

The cell-specific distribution and the seasonal variation of VIP- and preproVIP-like peptides suggests that they may act as transmitters or modulators in the nervous system and may be involved in the physiological adaptation of central neurons during long-term resting periods of snails.

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805.11

The number of striatal neurons with CCK-like immunoreactivity is ipsilaterally increased in unilateral 6-OHDA lesioned rats

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The existence of striatal CCK-ergic neurons is controversial discussed in literature. There is evidence both for striatal perikarya with CCK-like immunoreactivity (CCK-IR) and CCK mRNA in this rat brain region. Other studies failed to demonstrate perikarya with CCK-IR or CCK transscription material in basal ganglia of the rat brain. Previously, we have described a new avian antibody (Ab) raised against the sulphated CCK-8. Using this antibody neurons of different size and shape can be visualized in the striatum. Since CCK and dopamine (DA) were found to be colocalized in neurons of the mesencephalon of rats, it was speculated that CCK may have a neuromodulatory influence on DA. The 6-OHDA-lesion is a tool used commonly to investigate the influence of any neuronal substance on the DA function.. Thus, using the new avian anti CCK-Ab the present study was aimed to investigate whether or not a 6-OHDA (MFB) lesion has any influence on the distribution pattern of striatal CCK-IR structures.

It could be found that the number of CCK-ergic neurons significantly is increased ipsilaterally (150 %) to the lesioned hemispere 3 months following the 6-OHDA-lesion. No differences could be observed between the both hemispheres 1 month after the lesion. The increasing number of visualized CCK-ergic neurons seen in the lesioned hemisphere is interpreted as a functional parallel with receptor up/down regulation. The observed difference between both hemispheres may reflect a quite similar phenomenon but on a different level of functional brain organization. This response may also due to compensatory mechanisms which develop time dependently. Despite of our limited insight in neuronal interactions in general, the present results further substantiate the hypothesis of a nigrostriatal (and vice versa) regulatory CCK/DA relation (directly or indirectly).

This study was supported by the Deutsche Forschungsgemeinschaft (Scha 558/1-1) and the Bundesministerium für Bildung und Forschung (0310124A).

805.12

THE ORIGIN OF CCK IN CAUDATE AND NUCLEUS ACCUMBENS: COMBINED MICRODIALYSIS, RETROGRADE TRACING AND IN SITU HYBRIDIZATION ANALYSES.

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Contrary to several anatomical reports we have been unable to provide evidence for a dopaminergic midbrain origin for caudate (CPu) and nucleus accumbens (Acb) extracellular CCK measured by microdialysis (Soc. Neurosci. Abstr. **18**, 1369, 1992). Morino et al (1992) provided evidence for a cortical origin of extracellular CCK in the CPu. The current experiments were carried out to substantiate these findings and to extend them to the Acb using both microdialysis and histochemical approaches. Male Sprague Dawley rats were anaesthetized with Halothane. Kainic acid was injected bilaterally into a wide cortical area (total of 32 1ul injections). 10 days later rats were implanted with dialysis probes in CPu and Acb. After a 2h equilibration period samples were collected every 30 min. CCK release was evoked by incorporation of veratridine (50 µM) in the perfusion medium for 10 min and measured by RIA. Evoked release of CCK was reduced to 6% of naive control rats. Rhodamine-labeled latex beads were injected into the medial CPu or Acb shell. In situ-histochemical detection of CCK mRNA expression was achieved using a ^{35}S-labeled synthetic oligonucleotide probe. Retrograde tracing analysis revealed ipsi- and contra-lateral input from many regions of the neocortex and substantia nigra compacta (SNC). Also a massive projection from the medial and central amygdala, as well as the anterior dorsal and midline nuclei of the thalamus was observed. About 90% of the retrogradely labeled neocortical neurons also expressed CCK mRNA while this co-localization could be found only occasionally in the amygdala, SNC and thalamus. While very few retrogradely labeled neurons were found in the neocortex following Acb injection, several cortical amygdaloid nuclei, as well as the ventral part of the subiculum (S) and the ventral tegmental area (VTA) showed large numbers of ipsilateral projection neurons. Those in S and VTA were also found to express CCK.

805.13

IMMUNOLOCALIZATION AND QUANTITATION OF FIBROBLAST GROWTH FACTOR RECEPTOR-1 IN THE HUMAN PERIPHERAL NEUROMA.

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Immunocytochemical localization of fibroblast growth factor receptor-1 (FGFR-1) was studied in the cultured cells (passage 4 or 5) from human neuroma tissue obtained at the time of surgery. The cultured cells consisted of large numbers of fibroblasts and a few Schwann cells identified by anti-NGF receptor. The results showed that the fibroblasts were positively stained using an immunohistochemical procedure with a monoclonal antibody to recognize FGFR-1. Further, the subcellular location of FGFR-1 in the cells was investigated by electron microscopy using pre-embedding indirect immunogold double labeling. The particles (15 nm gold probe for FGFR-1) were found to be arranged regularly along the cell membranes at an average distance of 1.3±0.5 µm from the receptors, and particles (5 nm gold probe for basic fibroblast growth factor) were also present in the cytoplasm. It is interesting that the numbers of gold particles (for FGF receptor-1) were dramatically reduced after administration of basic fibroblast growth factor in the binding test. FGF membrane receptors were quantitated by flow cytometry using microbeads as the standard. The receptor density was calculated to be about 5600 per cell. The results will be useful for determining the function of FGF receptors in the formation of neuromas. Supported in part by DAMD17-93-V-3013.

805.14

DISTRIBUTION OF IMMUNOREACTIVE NERVE GROWTH FACTOR IN THE BRAIN AND PITUITARY GLAND OF *XIPHOPHORUS*.

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Antisera generated against Xiphophorus neurotropin-6 was utilized to study the distribution of immunoreactive (ir)- nerve growth factor (NGF) in the brain and pituitary gland of *Xiphophorus maculatus*, the platyfish, at different stages of sexual development. In both immature and mature animals, ir-NGF was found in an intensely stained cluster of perikarya in the anterior lateral nucleus lateralis tuberis (NLT). Processes of these cell bodies extended ventromedially toward and through the infundibulum and into the neurohypophysis of the pituitary gland. In the pituitary, immunoreactivity was seen in the pars intermedia and in the gonadotropic zone of the caudal pars distalis. Immunoreactivity was also noted in the hindbrain of mature animals. In neonatal animals, ir-granules were also noted around cells of the anterior olfactory lobe. These data suggest that NGF is involved in pituitary gland physiology, from very early in neonatal life, into sexual maturity. [Supported by NASA (NAGW-1704) and BARD (IS-2149-92)]

805.15

A COMPREHENSIVE ANALYSIS OF THE DISTRIBUTION OF FGF-2 AND FGFR1 IN THE RAT BRAIN. A.M. Gonzalez*, M. Berry†, P.A. Maher, A. Logan§, and A. Baird. The Scripps Research Institute, Dept. of Cell Biology, La Jolla, CA; †UMDS (Guy's Campus) London, UK; §Dept. of Clinical Chemistry, Univ. of Birmingham, UK

We examined the distribution of basic fibroblast growth factor (FGF-2) and its receptor (FGFR1). The results confirm the widespread distribution of both, and different regions express distinct patterns of protein: neuronal and non-neuronal cells show different subcellular distributions that vary according to the area where they are located. The intensity of staining and hybridization also varies according to the loci examined and the cell type involved. Astrocytes contain the highest levels of FGF-2 and FGFR1 mRNAs, and possess high levels of nuclear ir-FGF-2. Amongst non-neuronal cells, oligodendrocytes do not contain significant levels of ir-FGF-2; however they do express FGFR1 mRNA. In these cells, ir-FGFR1 is mainly associated with the myelin sheaths of neuronal fibers. In ventricular systems, ependymal cells synthesize and contain ir-FGFR1, but only cells lining the lateral wall of the III ventricle express FGF-2 mRNA. Subependymal cells contain high levels of both ir-FGF-2 and ir-FGFR1. Neurons express low levels of FGF-2 mRNA and ir-FGF-2 is localized predominantly to the perikaryon. However, select populations of neurons, (CA2 field of the hippocampus) show high levels of FGF-2 mRNA, in which the nucleus is strongly immunopositive. Similarly, high levels of FGFR1 mRNA are localized to select populations of neurons, (*e.g.*, amygdala). ir-FGFR1 is mainly associated with myelinated fiber tracts (*e.g.*, striatum), and some neurons show immunoreactivity in the perikaryon (*e.g.*, hippocampus), the nucleus (*e.g.*, mesencephalic trigeminal nucleus), or in axonal projections (*e.g.*, hypothalamus). In many areas, FGF-2 and FGFR1 mRNA and/or their translated protein do not co-localize in neurons (*e.g.*, neo-cortices) or even in the same regions of the brain (*e.g.*, substantia nigra). In other instances, mRNAs colocalize (*e.g.*, supraoptic nucleus). The highly specific anatomical distribution of ir-FGF-2 supports the notion that it plays a multifunctional role in the CNS. This comprehensive analysis should resolve numerous disparate findings described in the literature.

805.16

DIFFERENTIAL EXPRESSION OF CHROMOGRANIN A (CgA) HIGHLIGHTS ASTROCYTIC HETEROGENEITY. D.G.Munoz* and D.X. Deng. Dept. Pathology, University of Western Ontario, London, Ontario, Canada N6A 5C1

The neuropeptide CgA is subjected to cell type-specific proteolytic processing in many varieties of endocrine cells and neurons. McAuliffe and Hess reported that rat Bergmann glial cells are labelled by the antihuman CgA monoclonal antibody LK2H10. We now rule out cross-reactivity by demonstrating that LK2H10 does not recognize in western blots of rat brain any protein not present in adrenal medulla. We also immunostained the entire brains of adult male Sprague-Dawley rats with LK2H10 and polyclonal antibodies to the C-terminal sequence of rat CgA, and rat pancreastatin, a CgA-derived peptide. All three antibodies labelled Bergmann glia, as well as a subset of astrocytes. While all extracerebellar astrocytes expressing C-terminus (representing uncut CgA) and pancreastatin were restricted to the white matter, LK2H10 labelled a much wider population, including in addition astrocytes in the motor nuclei of cranial nerves, the molecular layer of cerebral cortex, the posterior (but not anterior) hypothalamus, and the stratum lacunosum-moleculare (but not radiatum) of hippocampus. Colocalization in the same cells of vesicular CgA and GFAP was confirmed by confocal microscopy. These observations show that astrocytes express CgA, and that region-specific populations differentially process CgA, suggesting a remarkable degree of functional heterogeneity, which may be related to CgA's modulatory actions on dendritic growth.

805.17

DIFFERENTIAL PROTEOLYTIC PROCESSING OF CHROMOGRANIN A IN RAT BRAIN NEURONS. D.X. Deng, C. Gibson* and D.G. Munoz. Dept. Pathology, University of Western Ontario, London, Ontario, Canada N6A 5C1

Chromogranin A (CgA) is a prohormone subjected to proteolytic processing. Cell type-specific differential expression of CgA-derived peptides has been observed in endocrine cells. To investigate whether differential processing of CgA takes place in neurons the entire brains of male Sprague-Dawley rats were immunostained with sequence-specific polyclonal antibodies to the CgA-derived peptides ß-granin and pancreastatin, and the C-terminus of CgA, and with 2 monoclonal CgA antibodies. Immunoreactivity for the C-terminus, representing uncut CgA, was widespread in perikarya, except for most neurons in cerebral neocortex, thalamus, neostriatum, and cerebellar cortex. All other antibodies labelled partially overlapping subsets of this population, and of synaptic terminals. For example, LK2H10 intensely labelled perikarya in the supraoptic and paraventricular nucleus, and ß-granin the central nucleus of the amygdala. In addition ß-granin was expressed in a subset of myelinated axons in the brain stem, and pancreastatin in circumventricular and subpial varicose fibers. These results indicate that CgA is proteolytically processed in neurons prior to axonal transport of large dense core synaptic vesicles. The differential expression of CgA fragments in neuronal populations suggests distinct functional roles for each CgA-derived peptide.

PEPTIDES: PHYSIOLOGICAL EFFECTS IV

806.1

INCREASED VASOPRESSOR ACTIONS OF NEUROPEPTIDE Y-(13-36) IN SPONTANEOUSLY HYPERTENSIVE VERSUS NORMOTENSIVE RATS MAY BE DUE TO INCREASES IN Y_2 RECEPTOR BINDING IN THE NUCLEUS TRACTUS SOLITARIUS. P. B. Hedlund*, J. A. Aguirre[1], J. A. Narváez[1], and K. Fuxe. Dept. of Neuroscience, Karolinska Institutet, S-171 77 Stockholm, Sweden and [1]Dept. of Physiology, Univ. of Málaga, Málaga, Spain

The C-terminal NPY fragment (13-36) [NPY-(13-36)], a NPY Y_2 receptor agonist, elicits vasopressor responses upon central administration. We have studied the cardiovascular responses of NPY-(13-36) and the distribution of NPY receptor subtypes within the nucleus tractus solitarius (nTS) in spontaneously hypertensive rats (SHR). NPY-(13-36) was injected intracerebroventricularly in different doses (7.5 to 3000 pmol) in awake, unrestrained rats to evaluate the cardiovascular effects. NPY receptor subtypes were studied by autoradiography using [^{125}I]peptide YY ([^{125}I]PYY) as a radioligand and by masking the Y_1 and Y_2 receptor subtypes with unlabelled [Leu^{31},Pro^{43}]NPY and NPY-(13-36) respectively. In both male SHR and age-matched male normotensive Wistar-Kyoto rats (WKY) NPY-(13-36) injections elicited vasopressor effects. In WKY this effect was dose-dependent and became significant at doses from 75 pmol, whereas in the SHR the vasopressor effect had a longer duration than in the WKY and became significant at lower doses (25 pmol) but associated with the development of an early ceiling effect. The heart rate was unaffected in both groups of rats. Total specific [^{125}I]PYY binding in the nTS was 25% higher in SHR than in WKY rats. By masking the Y_1 and Y_2 receptor subtypes respectively it could be shown that this difference was due to an increase in Y_2 receptor binding within the nTS. The present results give evidence for an increased potency but not an increased efficacy of NPY-(13-36) in inducing a pressor response in the SHR associated with a longer duration as compared with the WKY rats. These enhanced vasopressor effects may partly be explained on the basis of an increased density of Y_2 receptor (vasopressor effects) versus Y_1 receptor subtypes (vasodepressor effects) leading to a dominance of Y_2 over Y_1 transduction in the SHR. The peak activity of NPY-(13-36) in SHR may not be increased due to the already high blood pressure levels in these rats.

806.2

NEUROPEPTIDE Y mRNA IS INCREASED IN THE ARCUATE AND HIPPOCAMPAL HILUS AFTER ACUTE STRESS. C.D. Conrad* and B.S. McEwen. The Neuroendocrinology Lab, The Rockefeller University, New York, New York 10021

Arcuate and hilar NPY mRNA expression has been shown to be regulated by adrenalectomy (ADX) and a 1 week infusion of adrenal steroids (Wantanabe et. al., Mol Brain Res. (1995) 28: 135-140). ADX caused an increase in NPY mRNA whereas NPY mRNA decreased in the arcuate. We investigated whether acute stress would also exert a differential effect on NPY mRNA expression in the hippocampal hilus and arcuate nucleus. Male rats were acutely stressed by placing them in wire mesh restraints for 1 hour. Rats were decapitated at 0 (no stress), 1, 6 and 24 hrs after restraint. In situ hybridization was used to determine NPY mRNA levels. Film analysis of the autoradiograms indicated that NPY mRNA increased in the arcuate 24 hrs after one exposure to restraint stress, $F(3,35)=3.427$, $p<0.05$. NPY message was not elevated 1 and 6 hours after restraint compared to non stressed rats (10 rats/group). NPY mRNA increased in the dentate hilus 6 and 24 hrs after restraint compared to the non stressed and 1 hr groups, $F(3,19)=3.747$, $p<0.05$ (5-6 rats/group). Preliminary data from grain count analysis concur with the film results. Corticosterone (CORT) levels indicated that serum CORT was elevated immediately following the stressor (1 hr rats = 41.6 μg/dl) and that these levels declined by 6 hours. These data indicate that exposure to one hour of restraint stress is sufficient to elevate NPY mRNA levels in both the arcuate nucleus and hippocampal hilus. Furthermore, acute stress did not differentially alter NPY mRNA expression between the arcuate and hilus. Supported by grants MH10804 to CC and MH41256 to BM.

806.3

EFFECTS OF NEUROPEPTIDE Y AND SIGMA RECEPTOR ANTAGONISTS ON NMDA-STIMULATED [^{3}H]DA RELEASE FROM SLICES OF RAT PREFRONTAL CORTEX AND STRIATUM. D.T. Ault* and L.L. Werling. Depts. of Neuroscience and Pharmacology, The George Washington University Medical Center, Washington, DC 20037.

Although the identity of the endogenous ligand for sigma receptors is controversial, various neuropeptides and steroids have been shown to compete for binding to these receptors. Among endogenous sigma receptor ligand candidates is neuropeptide Y (NPY) which mimics electrophysiological preparations of some sigma ligands. We have previously shown that prototypical sigma agonists such as (+)pentazocine and BD737 inhibit stimulated [^{3}H]DA release in various brain regions. Using a superfusion system, we compared the effect of NPY on stimulated [^{3}H]DA release to the effects of these ligands. In contrast to (+)pentazocine- and BD737-mediated inhibition of release, NPY enhanced release at nM concentrations. Several sigma antagonists reversed the enhancement. Effects of NPY receptor antagonists are currently being tested. (Supported by a NIDA grant to LLW.)

806.4

BLOOD PRESSURE CHANGES INDUCED BY ANGIOTENSIN ANALOGUES DESIGNED TO INTERACT WITH THE AT$_1$ RECEPTOR SUBTYPE. J.W. Wright*, A.J. Bechtholt, S.L. Esser and J.W. Harding. Departments of Psychology and VCAPP, Washington State University, Pullman, WA 99164-4820.

Three angiotensin receptor subtypes have now been identified and characterized within the brain renin-angiotensin system (RAS). Of these, the AT$_1$ subtype appears to be responsible for mediating the classic physiologies and behaviors associated with the brain RAS, namely pressor and drinking responses, vasopressin release, salt appetite, and sexual behavior accompanied by cyclic regulation of reproductive hormones. It has been assumed that angiotensin II (AngII) activates the AT$_1$ receptor subtype, although there is evidence that angiotensin III (AngIII) may also act at this site (reviewed by Chiu et al., 1993). The present investigation utilized three metabolically stable angiotensin analogues in an effort to determine the form of angiotensin that activates the centrally mediated pressor response. Each of these analogues was modified at the N-terminus with a hydroxylethylamine group ($CHOHCH_2NH$) amide bond. This bond primarily differs from the peptide bond in that it permits free rotation of the backbone bonds, it is 1.67 angstroms longer, and it significantly increases the half-life of each molecule.

Intracerebroventricular infusion of each analogue (100 pmol/min) for 10 min in alert rats indicate maximum mean (+SEM) systemic blood pressure elevations of 20+5.7, 21+2.6, and 17+0.9 mm Hg for AngII, III, and IV pseudopeptide analogues, respectively. Comparable values for native AngII, III, and IV were 24+2.2, 21+3.1, and 15+1.1 mm Hg. These results suggest that native AngII and III are equipotent at the AT$_1$ receptor subtype, while AngIV is less potent. The advantage offered by AngII over AngIII may be that it activates this receptor as AngII, and is then converted to AngIII that also activates the receptor, followed by conversion to AngIV which is briefly activated. AngIII does not offer this "multiple-ligand" effect.

806.5

THE EFFECTS OF ESTROGEN ON ANGIOTENSIN II STIMULATED PROLACTIN SECRETION IN VITRO. W. Bryant, K. Zechiel and P. Callahan*, Dept of Zoology, Center for Neuroscience, Miami University, Oxford, Ohio 45056.

The role of Angiotensin II (AII) in the physiological regulation of Prolactin (PRL) release is unknown. The purpose of these studies was to determine the effect of estrogen on the AII - induced PRL secretory response from dispersed anterior pituitary cells. Female rats were ovariectomized (ovx) at 4-6 weeks of age and divided into 2 groups. One group of ovx females received estrogen replacement pellets (ovx + E), while a second group received placebo (ovx). A third group of female rats was left intact. Two months later, the stimulatory effects of AII on PRL release were determined *in vitro*.

Administration of AII significantly stimulated PRL release in cells obtained from intact female donors. AII did not stimulate PRL secretion from cells obtained from ovx + E treated females and was only weakly stimulatory to cells obtained from ovx rats. These results indicate that estrogen alone is not responsible for the sensitivity to AII stimulation observed in cells from intact female rats. Also, AII did not stimulate PRL secretion from cells obtained from male rat donors. Scatchard analysis of Sar^1-Ile^8 AII binding revealed no difference between K_d and B_{max} values in male and female anterior pituitaries.

Thus, it appears that anterior pituitary cells from female rats are sensitive to AII stimulation, but that estrogen is not solely responsible for this sensitivity. This response to AII stimulation does not appear to be a receptor mediated phenomenon since there was no difference in receptor binding characteristics (K_d and B_{max} values) between pituitaries obtained from male or female rats. (Supported by NIH grant DK 48023 to PC).

806.6

GLUCOCORTICOID REGULATION OF THE VASOPRESSIN V1a RECEPTOR IN THE SEPTUM OF RAT BRAIN

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Vasopressin is intimately involved in the hypothalamo-pituitary adrenal axis. V1a vasopressin receptors (V1aR) , the primary subtype expressed in the brain, and their mRNA, are expressed in WRK-1 mammary tumor cells. Both the receptor and its mRNA are enhanced by dexamethasone (DEX) treatment. To examine the molecular basis of this effect, we have isolated a genomic clone encoding the rat V1aR. Approximately 3.5kb have been sequenced revealing the presence of 3 putative glucocorticoid response elements (GREs) in the 5' flanking region of the gene. Gel mobility shift assays using these putative GREs and nuclear extract from DEX treated WRK-1 cells indicate that 2 of the 3 putative GREs are active in protein binding and might mediate transcriptional effects of glucocorticoids. The septum of the rat brain expresses a vasopressin V1aR which has been impicated in various rodent behaviors. In order to examine in vivo effects of adrenal steroids on septal V1aRs, we bilaterally adrenalectomized (ADX) male rats and hormone replaced them with either DEX in different concentrations, or with aldosterone. The effects were evaluated in the septum of these animals using a radiolabelled specific V1aR antagonist ^{125}I Sar^7-AVP. ADX significantly decreases ($p<0.05$) V1aR binding site density below those of the sham ADX controls. Additionally, DEX, but not aldosterone, was able to restore V1aR binding in ADX animals to levels comparable to those of sham controls. Studies are currently in progress to determine the effects of these steroids on V1aR mRNA in the septum. (Supported by NS20311, the VA and Pharmacological Sciences Training Grant 670489).

806.7

PHYSICAL AND EMOTIONAL STRESSORS STIMULATE THE RELEASE OF VASOPRESSIN WITHIN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS OF RATS: A MICRODIALYSIS STUDY. C. T. Wotjak, M. Kubota, M. Engelmann, I. Neumann and R. Landgraf. Max Planck Institute of Psychiatry, Clinical Institute, D-80804 Munich, Germany.

The neuropeptide arginine vasopressin (AVP) is thought to play an important role in HPA axis regulation at the level of the paraventricular nucleus (PVN). The release of AVP within the PVN in response to physical and emotional stressors was monitored by microdialysis in adult Wistar rats. Compared to pre-stress values (0.1-1.0 pg/30-min dialysate), swimming in the Morris water maze increased AVP release (380%, $p<0.01$, n=8). Intra-PVN release of the neuropeptide peaked also during social defeat (226%, $p<0.01$, n=11), but was just slightly increased during exposure to "milder" stressors (exposure to a juvenile: 160%, n.s.; novel environment: 137%, n.s., n=8 each). These data provide evidence that both physical and emotional stressors are potent to evoke intranuclear AVP release and confirm the hypothesis of an involvement of the intracerebrally released neuropeptide in coping with intense stress. Since plasma corticosterone demonstrated a substantial activation of the HPA axis in response to all stressors used (Morris water maze: 574%, $p<0.01$, n=6; social defeat: 510%, $p<0.01$, n=6; juvenile exposure: 250%, $p<0.01$, n = 10; novel environment: 424%, $p < 0.01$, n = 6) further studies have to investigate to which extent AVP release within the PVN contributes to the regulation of the HPA axis activity. Supported by VW.

806.8

SPINAL CORD OXYTOCIN MEDIATES THE PUPIL DILATATION RESPONSE TO VAGINOCERVICAL STIMULATION IN THE RAT. G. Sansone*, E.V. Kruck, M. Ganduglia-Pirovano and B.R. Komisaruk. Institute of Animal Behavior, Rutgers The State University of New Jersey, Newark, N.J. 07102.

The present study was designed to investigate whether oxytocin (OT), which is released into the spinal cord after vaginocervical stimulation (VS), mediates responses to VS. Ovariectomized, estrogen-primed (10 µgEB/100 g bw, SC x 3 d) rats were used. OT (10 and 25 µg in 5 µl saline) was administered intrathecally (i.t.) at the lumbar level and all rats were tested for responses to VS (i.e. facilitation of lordosis, analgesia, pupil dilatation, increase in blood pressure and heart rate). A selective OT receptor antagonist [d(CH_2)$_5$-Tyr(Me)^2-Thr^4-Tyr-NH_2^{9}] OVT (OTA, 25 µg in 5 µl saline) was then administered i.t. and VS (300 g force) was applied to the cervix to determine which responses, if any, previously mimicked by i.t. OT, could be blocked. RESULTS: Administration of OT i.t. (10 or 25 µg) produced a significant increase in pupil diameter (PD) within 1 min ($p < 0.05$, ANOVA). Significant PD persisted for 20 min (10 µg OT) and for 25 min (25 µg OT) post-injection, respectively. When OTA was injected i.t., the magnitude of the increase in PD in response to VS compared to the saline control was significantly reduced within 3 min post-injection ($p < 0.05$, ANOVA). The antagonistic effect of OTA was still present 60 min post-injection ($p < 0.05$, ANOVA). No effect of OTA alone was observed. OT also significantly increased blood pressure ($p = 0.001$, ANOVA); however, administration of OTA did not antagonize the hypertensive effect of VS. No change in heart rate, analgesia, or lordosis was induced by either OT or OTA. CONCLUSION: The present findings suggest that OT is a central nervous system transmitter that mediates specifically VS-produced PD by stimulating the autonomic (sympathetic division) outflow from the spinal cord. Support: NIH - Fogarty International Center - 1RO3-TW00394 (BRK).

806.9

OXYTOCIN ANTISENSE REDUCES SALT INTAKE IN THE BARORECEPTOR DENERVATED RAT. M. Morris*, P. Li, C. Barrett, M.F. Callahan. Dept. of Physiology and Pharmacology and The Hypertension Center, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157

Interruption of baroreceptor input to the brain produces a state of salt sensitivity. In view of the role of oxytocin (OT) in the control of salt intake and excretion, experiments were performed to evaluate the effect of central injection of OT antisense oligomers on reduced salt intake shown in sinoaortic denervated rats (SAD). Paraventicular injection of OT antisense (AS) decreased intake of 2% NaCl in the SAD, but not in the sham operated (SO)controls. The SAD rats consumed 22±4 ml after mixed base control injection as compared to 8±4 ml after the OT AS injection ($p < 0.05$). There was no difference in the response of the SO controls. The SAD animals demonstrated an increase in the plasma OT response to 24 hours salt loading (3.2±0.7 to 6.9±0.8 pg/ml as compared to 2.8±0.9 to 4.4±0.9 pg/ml, SAD vs SO). The increased endocrine response occurred even though salt intake was lower in the SAD animals. There were no group differences in plasma electrolytes or posterior pituitary OT content. Results demonstrate a specific effect of OT antisense in the denervated animal, suggesting that central OT stimulates salt intake in this model.

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806.10

EFFECT OF CENTRAL NEUROTENSIN ADMINISTRATION ON FOS EXPRESSION IN THE RAT BRAIN - RELATING FUNCTION TO ANATOMY. T.D. Ely*P.D. Lambert, R. Gross, C.B. Nemeroff and C.D. Kilts. Dept. of Psychiatry, Emory Univ. Sch. of Med., Atlanta, GA 30322.

The central administration of the neuropeptide neurotensin (NT) is associated with robust behavioral, neuroendocrine and hypothermic responses. The neural substrates and sites of NT-induced alterations in brain function are unclear. The expression of the immediate early gene c-fos has been described as a monitor of neuronal activation in response to a range of stimuli. We have used immunohistochemistry to localize cells activated by the intracerebroventricular (icv) administration of NT and attempted to correlate brain regional Fos responses with endocrine or hypothermic effects to determine related sites of action. Groups of male Sprague-Dawley rats (n=5) received single icv injections of artificial CSF or NT (0.3, 3 or 30μg). 90 minutes following injection, brains were removed, 40μm slices were cut and stained for Fos. NT administration produced a dose-dependent hypothermia, increase in serum corticosterone and increase in the number of Fos-positive cells within the central (Ce) and basolateral nuclei of the amygdala and the paraventricular and supraoptic hypothalamic nuclei. Injection (i.p.) of the selective NT antagonist SR48692 30 minutes before icv injection of NT completely abolished the increase in corticosterone and attenuated the increase in Fos-positive cells within the amygdala. A strong correlation was found between the number of Fos-positive cells in the Ce and the serum corticosterone level for each animal tested (n=16, r=0.72). These data show that central NT activates neurons within the amygdala and the hypothalamus and that the effect of NT to increase serum corticosterone is mediated via an action involving the Ce. Our data further suggests the existence of multiple central NT receptor subtypes.

806.11

ASTRESSIN, A POTENT CYCLIC CRF ANTAGONIST J. Rivier, J. Gulyas, M. H. Perrin, S. C. Koerber, S. Sutton, A. Corrigan, S. L. Lahrichi, A. G. Craig, R. Evans*, W. Vale and Catherine Rivier. The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.

Predictive methods, physicochemical measurements (NMR, CD) and structure activity relationships studies suggest that corticotropin-releasing factor (CRF) and its family members (urotensins and sauvagine) assume an α-helical conformation when interacting with the CRF receptor(s). We have scanned the r/hCRF sequence with several constraining modalities such as an i-(i+3) and i-(i+4) bridge consisting of the D or L-Glu-Xaa-Xaa-(Xaa)-D or L-Lys scaffold. From this series we have identified a unique substitution that resulted in an antagonist, Astressin (a 30 -peptide) that is >100 times more potent than the α-helical CRF_{9-41}, 42 times more potent than [DPhe^{12},Nle^{21,38}]r/hCRF_{12-41}, our present standard, and 400 times more potent than the corresponding linear analog in an *in vitro* pituitary cell culture assay. As expected, Astressin had very low affinity for the CRF binding protein and very high binding affinity in a specific receptor assay. *In vivo*, Astressin is significantly more potent and longer acting than any previously tested antagonist at inhibiting basal or stimulated ACTH secretion. Indeed, Astressin blocked stress-induced ACTH release at less than 0.3 mg/kg, iv in the rat. In an agonist (a 38-peptide), this structural modification resulted in a 2-fold increase in potency as compared to the parent linear analog. The differences in relative potency in the agonist (2-fold loss) and antagonist (400-fold loss) series between the linear and cyclic forms suggest that the bridge reinstates a structural motif in the antagonist equivalent to that brought about by an unaltered N-terminus octapeptide in the agonist.

CATECHOLAMINE RECEPTORS: GENETICS

807.1

THE STATUS OF THE DOPAMINE D_2 RECEPTOR (DRD2) LOCUS AS A SUSCEPTIBILITY FACTOR IN AUTISM: FAMILY ASSOCIATION STUDIES. S.D. Flanagan*[1], E. Cook[2], E. Courchesne[3], A. Lincoln[3], C. Lord[2], B. Leventhal[2], R. Gysin[1] and R. Courchesne[3]. [1]Beckman Res. Inst. of the City of Hope, Duarte, CA; [2]University of Chicago Hospitals, Chicago, IL; and [3]Children's Hospital, San Diego, CA.

Case-control studies support the hypothesis that a genetic variant at the DRD2 locus contributes significantly to risk for autism. The hypothesized DRD2 susceptibility factor is neither necessary nor sufficient to cause autism and probably interacts with other factors, genetic or non-genetic. Linkage disequilibrium with the DRD2 *Taq*A1 allele is the subject of considerable controversy concerning its role as a risk factor in severe alcoholism. Much of the controversy revolves around limitations inherent in any genetic association study utilizing the case-control design. In severe alcoholism, there is the additional problem that variations in subject recruitment could select for alcoholics possessing different risk factors, explaining the wide divergence in various reports. By contrast, autism as diagnosed by well established ADI and ADOS criteria, is unlikely to be affected by vagaries of subject recruitment; furthermore, the parents of autistic subjects are readily recruited providing the opportunity for the calculation of haplotype relative risk in the powerful family association design. We recruited 31 sets of autistic patients and their parents (Courchesne lab), and found a trend for elevated transmission of the DRD2 *Taq*A1 allele to probands (χ^2=3.05, p=0.08). Data for the DRD2 *Taq*B1 allele, which lies in strong linkage disequilibrium with the *Taq*A1 allele, were as follows: 12 B1 and 50 B2 parental alleles were transmitted to probands; 3 B1 and 59 B2 alleles were not transmitted (Fisher exact, one-tailed, p=0.01). We have now recruited a separate, replication sample of 27 sets of probands and parents (Cook lab) to test our hypothesis that the association of autism with DRD2 *Taq*B1 allele exists. *A portion of this work was supported by the Wacker Foundation.*

807.2

GENDER, HERITABILITY AND D_1 RECEPTOR BINDING. S. Kanes*, S. Sanderson, M. Silverman, E. Rasmussen, L. Cipp, K. Dains, B. Hitzemann, and R. Hitzemann. Departments of Psychiatry, Neurobiology and Pharmacology, SUNY at Stony Brook, NY 11794-8101.

The D_1 dopamine receptor specific binding of ^{125}I-SCH 23382 was determined in discrete brain regions of both male and female C57BL/6 (B6), DBA/2 (D2), B6♀D2♂ F_1 cross and F_2 cross mice. Data were obtained for the substantia nigra zona reticulata (SNr), the core and shell of the nucleus accumbens (NAc) and both the lateral and dorsomedial aspects of the caudate-putamen (CPu). N = 12/sex for the parental strains and the F_1 cross and 30/sex for the F_2 cross. Variance in receptor binding for the F_2 cross is derived from both heritable and non-heritable causes. The non-heritable or environmental component (V_E) was estimated from the variances seen in the parental strains and the F_1 cross. For the F_2 males, significant broad sense heritability was found in the NAc core (0.75) and the NAc shell (0.60). For the F_2 females, significant heritability was detected only in the NAc shell (0.71). In comparison to the males, V_E for the females was significantly higher in both the NAc core and the SNr; in the SNr, the difference in variability was > 300%. D_1 receptor binding was generally higher in the D2 strain, a difference (60%) which was most marked for the males in the NAc shell. The most marked gender effect was seen in the SNr; receptor binding for the B6 females was > 100% higher than that of the B6 males. The F_2 cross was genotyped for *D13Mit13*, a microsatellite which is polymorphic between the B6 and D2 strain and which is closely linked to *Drd1*. No association was detected between receptor binding and genotype.

807.3

GENDER, HERITABILITY AND D_2/D_3 RECEPTOR BINDING. L. Cipp*, S. Sanderson, M. Silverman, E. Rasmussen, J. Gatley, S. Kanes, K. Dains, B. Hitzemann, and R. Hitzemann. Departments of Psychiatry, Neurobiology and Pharmacology, SUNY at Stony Brook, NY 11794-8101.

The receptor specific binding of ^{125}I-epidepride was determined in discrete brain regions of both male and female C57BL/6 (B6), DBA/2 (D2), B6♀D2♂ F_1 cross and F_2 cross mice. Epidepride binds with a high affinity to D_2 and D_3 but not D_4 dopamine receptors. Data were obtained for the substantia nigra zona compacta (SNc), the ventral tegmental area (VTA), the core and shell of the nucleus accumbens (NAc) and both the lateral and dorsomedial aspects of the caudate-putamen (CPu). The binding of ^{3}H-7-hydroxy-DPAT was used to determine that D_3 receptor binding is < 5% of the total epidepride binding in all brain regions except the NAc. In general, there was no difference in the heritability of receptor binding between F_2 males and females. Collapsing the data across gender, the broad sense heritability was SNc (0.56), VTA (0.50), NAc core (0.52), NAc shell (0.62), dCPu (0.56) and lCPu (0.60). In the NAc core and shell, binding was modestly higher (10-25%) in the B6 as compared to the D2 strain. Further, compared to the B6 males, the B6 females showed a significantly higher receptor binding in the core (+25%). In both the SNc and VTA, receptor binding was markedly (> 50%) higher in the D2 strain; there were no gender effects in these regions. The F_2 cross (N=100) was genotyped for *D9Mit22*, *D9Mit4* and *D9Mit21*, microsatellites which are polymorphic between the B6 and D2 strains and which are closely linked to *Drd2*. The results show that for both the striatum and midbrain, the D2 allele is associated with higher receptor density.

807.4

GENDER, HERITABILITY AND DOPAMINE TRANSPORTER (DAT) BINDING. E. Rasmussen*, S. Sanderson, M. Silverman, L. Cipp, B. Hitzemann, and R. Hitzemann. Departments of Psychiatry, Neurobiology and Pharmacology, SUNY at Stony Brook, NY 11794-8101.

The DAT specific binding of ^{125}I-RTI 55 was determined using quantitative receptor autoradiography in the nucleus accumbens (NAc) core and shell of both male and female C57BL/6 (B6), DBA/2 (D2), B6♀D2♂ F_1 cross and F_2 cross mice. N = 12/sex for the parental strains and the F_1 cross and 30/sex for the F_2 cross. Variance in receptor binding for the F_2 cross is derived from both heritable and non-heritable causes. The non-heritable or environmental component (V_E) was estimated from the variances seen in the parental strains and the F_1 cross. For the F_2 males, significant broad sense heritability was found in the NAc shell (0.80) and the NAc core (0.75). For the F_2 females, significant heritability was detected only in the NAc shell (0.76). There were no significant differences in V_E between males and females in either brain area. In the NAc shell, DAT binding was significantly higher in the D2 as compared to the B6 strain; this difference was most marked for the females (+ 57%). The shell F_1 and F_2 data suggest that the B6 genotype is dominant but that significant epistatic effects are also present i.e. the F_2 phenotype is significantly higher than would be predicted on the basis of a simple dominance/recessive model. The strain differences in the core are less marked and only significant for the males (D2 > B6). DAT binding is significantly higher for females in both brain regions. In the core the difference is most marked for the B6 strain (+ 36%) and in the shell for the D2 strain (+ 63%). DAT binding is markedly higher in the core; collapsing across strain and gender, the average difference is 125%.

807.5

GENETICS AND THE ORGANIZATION OF CHOLINERGIC NEURONS IN THE MOUSE CAUDATE-PUTAMEN: CONFIRMATION OF TWO QTL. K.M. Dains*, B.A. Hitzemann and R.J.Hitzemann. Departments of Neurobiology and Behavior and Psychiatry and Behavioral Science, SUNY at Stony Brook, NY 11794.

The broad sense heritability of cholinergic cell density, as high as 78% in some areas of the caudate-putamen (CPu), was determined from the analysis of C57Bl/6J (B6) and DBA/2J (D2) inbred mouse strains and B6D2 F_1 and F_2 hybrid crosses. In order to identify genes associated with this phenotype, quantitative trait locus (QTL) analysis was performed using 25 strains of the BXD/Ty series. Confirmation of the putative QTL was accomplished using 50 B6D2 F_2 hybrids phenotyped for cholinergic cell number. Two possible candidate QTL were revealed by this analysis, *D9Mit21* on chromosome 9, near *Drd2*, the D_2 dopamine receptor gene, and *D12Mit7* on chromosome 12, near *c-fos*. *Drd2* is associated with neuroleptic-induced catalepsy and D_2 dopamine receptor density. *c-Fos* is induced by muscarinic agonists and antagonists as well as dopamine antagonists such as haloperidol. Further, as reported elsewhere at this meeting (Patel et al.), in comparison to the B6 strain, the haloperidol sensitive D2 strain shows a more marked Fos response in most regions of the striatum. Overall, the QTL associated with *D9Mit21* and *D12Mit7* are considered good candidates a) for additional confirmation in other F_2 crosses, b) for the formation of congenic lines and c) for eventual positional cloning of the relevant gene(s).

807.6

GENETICS AND THE HALOPERIDOL-INDUCED INCREASE OF FOS IN THE MOUSE STRIATUM. N. Patel, B. Hitzemann, and R. Hitzemann*. Departments of Psychiatry, Neurobiology and Pharmacology, SUNY at Stony Brook, NY 11794-8101.

Haloperidol and related antipsychotic drugs (those which induce extrapyramidal symptoms {EPS}) increase striatal Fos expression. The Fos response in the caudate-putamen (CPu) is blocked by anticholinergic drugs and markedly reduced after the administration of atypical antipsychotic drugs such as clozapine. These data support the notion that the Fos response is predictive of EPS. An alternative method to test this hypothesis is to compare the Fos response among inbred strains of mice which are differentially sensitive to haloperidol-induced catalepsy (catalepsy is the murine equivalent of EPS). The ED_{50} for haloperidol-induced catalepsy in the DBA/2 (D2) strain is 0.4 mg/kg and in the C57BL/6 (B6) strain is 4.0 mg/kg. Male D2 and B6 mice were administered 0.3 and 1.0 mg/kg of haloperidol, sacrificed 1 hour later and Fos expression was measured in the rostral striatum using standard immunocytochemical methods. In the lateral CPu, Fos expression in response to both doses was on average >100% higher in the D2 strain. The pattern was the same in the core of the nucleus accumbens (NAc) but the magnitude of the difference was less (ca. 50%). In the shell of the NAc, the difference between the D2 and B6 strains was the same as in the core at 0.3 mg/kg but began to reverse at 1.0 mg/kg. In the dorsomedial CPu and depending on the rostral/caudal level sampled, there was either no difference between strains or the B6 strain showed a greater Fos response. Thus, only in some brain regions does the Fos response parallel the behavioral differences in strain sensitivity.

SEROTONIN RECEPTORS: 5-HT$_3$

808.1

FUNCTIONAL AND BINDING STUDIES OF PHOSPHORYLATION AND GLYCOSYLATION SITE MUTANTS OF 5-HT$_3$ RECEPTORS: **E.J. Fletcher, M.I. Sepulveda, T. Green, R. Pinnock* and S.C.R. Lummis. Division of Neurobiology, LMB and Department of Zoology, University of Cambridge, Cambridge, UK..**

5-HT$_3$ receptors, members of the family of ligand-gated ion channels (Maricq et al.,1991), possess a number of potential phosphorylation and glycosylation sites. We have used site-directed mutagenesis of these sites to examine their function in homomeric 5-HT$_3$ receptors. In particular we have explored the function of phosphorylation in generating differences observed in the two splice variants of the 5-HT$_3$ receptor, which differ by 6 amino acids and a potential phosphorylation site. Full length 5-HT$_3$R-As (short) DNA was obtained from N1E-115 mRNA using PCR. Coding sequence for the additional 6 amino acids in the 5-HT$_3$R-A (long) subunit was inserted using site-directed mutagenesis, as were mutations to remove potential phosphorylation and glycosylation sites. Sequences were inserted into the eukaryotic expression vector pRc/CMV and transfected into HEK 293 cells using calcium phosphate precipitation. Mutants were characterised using radioligand binding and whole cell patch clamp electrophysiology. Radioligand binding studies with [^{3}H]granisetron showed that relative potencies of a selection of 5-HT$_3$ receptor selective ligands were similar (GR65630 > mCPBG > MDL72222 > 5-HT) in long, short and functional mutant receptors. However patch clamp studies showed differences in agonist affinity and efficacy (long > short); binding studies also distinguished between the splice variants: K_d = 0.38 ± 0.03 and 0.24 ± 0.02 (n=4) for long and short forms respectively. Phosphorylation was not responsible for this observed change in affinity. The results from the glycosylation studies show that only one of the potential glycosylation sites (N191) is crucial for ligand binding and function.

Maricq, A.V., Peterson, A.S. Blake et al. (1991) Science 254, 432-437

808.2

IDENTIFICATION OF AMINO ACID RESIDUES INVOLVED IN AGONIST/ANTAGONIST LIGAND RECOGNITION BY THE 5-HT$_3$ RECEPTOR. J.A. Steele, F.G. Boess†, L.J. Steward, M. Davies* & J.L. Martin. Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7; †F. Hoffmann-La Roche AG, Department PRPN, P.O. Box CH-4002, Basel, Switzerland.

The 5-HT$_3$ receptor is a member of the ligand-gated ion channel family. To investigate the importance of certain amino acids in agonist/antagonist ligand recognition, we have performed site-directed mutagenesis on the 5-HT$_3$-aL cDNA isolated from NG108-15 cells. 5-HT$_3$ receptor mutants were transiently expressed in HEK 293 cells and characterized using whole-cell patch clamp electrophysiology and radioligand binding.

The E106D mutation caused a 10-fold reduction in the Kd of [^{3}H]GR65630, while there was a 100-fold reduction in the Ki for 5-HT in competition with this ligand, compared to wild type. There was no change in the EC_{50} value for 5-HT measured with electrophysiology. However, the Ki and EC_{50} values for the E106N mutation were decreased 40-fold and 10-fold, respectively, when compared to wild type.

E106 appears to be particularly important in agonist recognition for the high affinity desensitized conformation of the receptor which is measured by binding, but less so for the low affinity resting conformation, which is measured by electrophysiology and is integral to channel activation.

Supported by Glaxo Canada and MRC Canada.

808.3

SOLUBILISATION OF THE 5-HYDROXYTRYPTAMINE$_3$ RECEPTOR RECOGNITION SITE EXPRESSED IN PIG CEREBRAL CORTEX

S Fletcher* & NM Barnes. Department of Pharmacology, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TT UK

5-Hydroxytryptamine$_3$ (5-HT$_3$) receptor recognition sites were solubilised from homogenates of pig cerebral cortex (1 g original wet weight/mL in 25 mM Tris, pH 7.45) by addition of an equal volume of buffer (25 mM Tris, 2 mM EDTA, 100 µM PMSF, 10 µg/mL bacitracin, 10 µg/mL sodium azide, 10 µg/mL soybean trypsin inhibitor, pH 7.45) containing 0-4% Triton X-100. Maximum yield (35.8% ± 3.2; % mean ± SEM, n=5) was obtained using Triton X-100 at a final concentration of 0.4%.

Preliminary studies suggest that the pharmacological profile of solubilised 5-HT$_3$ receptor sites is similar to that obtained for the receptor sites in the crude homogenates. For example, the antagonists ondansetron and tropisetron, and the agonist 5-HT, compete for [^{3}H]-(S)-zacopride binding to solubilised receptor preparations giving similar pKi values to those obtained in crude homogenate preparations; 8.09± 0.08, 7.33 ± 0.21 and 6.43 ± 0.12 (mean ± SEM, n=3) respectively, compared to values of 7.71 ± 0.16, 7.44 ± 0.05 and 6.28 ± 0.02 (mean ± SEM, n=3-6) obtained in crude homogenates.

We conclude that the 5-HT$_3$ receptor recognition site expressed in pig brain has been successfully solubilised, which is an essential prerequisite for purification of the receptor.

S. Fletcher is recipient of an AJ Clark Studentship from the British Pharmacological Society.

808.4

IN VIVO INHIBITION OF K$^+$-EVOKED ACETYLCHOLINE RELEASE BY 5-HT3 RECEPTOR ACTIVATION. P. Blandina, I. Ceccarelli, R. Corradetti*, G. Pepeu & M.G. Giovannini. Dipartimento di Farmacologia Preclinica e Clinica, Universitá di Firenze, Firenze, Italy

Although 5-HT3 receptors are present in the cortex and hippocampus, their physiological role is not clearly defined. We show here that 5-HT modulates the release of ACh from cortex and hippocampus of freely moving rats using the microdialysis technique. Perfusion flow rate was 3 µl/min. Twenty four hours after implantation with dialysis fiber, hippocampus or cortex of male Wistar rats (250 g) released spontaneously 2.5±0.1 pmol/10 min ACh (N=87), measured by HPLC with electrochemical detection. Two identical 100 mM K$^+$ stimulations, given through the dialisys fiber at a 90 min interval, each almost doubled ACh release. 5-HT (1-50 µM) inhibited K$^+$-evoked release of ACh up to about 50% from cortex and hippocampus. The 5-HT3 agonists phenylbiguanide (0.1-10 µM) and 1-m-Cl-phenylbiguanide (1-10 µM) mimicked 5-HT effect in cortex, but not in hippocampus. The effects of both 5-HT and phenylbiguanide in cortex were completely antagonized by the 5-HT3 antagonist ICS 205-930 (0.5 mg/kg s.c.), but not by methiotepin, antagonist at 5-HT1-like and 5-HT2 and not at 5-HT3 receptors. Thus, 5-HT3 receptors may contribute to the role of ACh in controlling the cortical but not the hippocampal functions.

808.5

5-HT$_3$ RECEPTOR AGONISTS REDUCE GLUTAMATE NEUROTRANSMISSION IN THE HIPPOCAMPUS OF THE RAT. K. Batsche*, and R.Y. Wang. Dept. of Psychiatry, SUNY Stony Brook, Stony Brook, NY, 11794.

We have previously shown that the 5-HT$_3$ receptor agonist 2-methyl-5-HT reduces both EPSP's and IPSP's of CA1 pyramidal cells evoked by stimulation of the Schaffer collaterals. This action is receptor specific and concentration dependent. However, others have suggested that activation of 5-HT$_3$ receptors in the hippocampus can increase GABA release at interneuron terminals. In the present study, we further examine the role of 5-HT$_3$ receptors in this area using the more selective and potent 5-HT$_3$ receptor agonist SR 57227A (SR), appplied either iontophoretically or via bath. Glutamatergic neurotransmission was elicited either by Schafffer collateral stimulation or local iontophoresis of glutamate. We used whole cell current- and voltage-clamp recordings from CA1 pyramidal neurons. Glutamate was pulsed with an ejecting current adjusted to produce short depolarizations of 7-15 mV. Similar to the action of 2-methyl-5-HT, SR consistently reduced PSP's elicited by Schaffer collateral stimulation without changing the membrane properties of the cells. Further, local iontophoresis of SR reduced glutamate-induced depolarizations to about 50% of control values, while not altering the kinetics of glutamate uptake or receptor activation or the input resistance of the cell. These actions of SR were blocked by the selective 5-HT$_3$ receptor antagonists BRL43694 and tropisetron but not the 5-HT$_{1A}$ receptor antagonist (+)-WAY100135. The 5-HT$_{1A}$ receptor agonist 8-OH-DPAT also inhibited glutamate-induced depolarizations, which was blocked by (+)-WAY100135. In several cells, SR produced a slow hyperpolarization of the cell's resting potential that did not desensitize. It appears from these experiments that 5-HT, acting at 5-HT$_3$ receptors, will modulate glutamatergic currents in the CA1 area of the hippocampus in a manner not directly involving the opening or closing of membrane ion channels. (Supported by USPHS grants MH41440 and DA07193; K.B. was supported by NRSA fellowship F31-DA05532)

808.6

POTENTIATION OF THE INHIBITORY ACTION OF DOPAMINE BY THE SEROTONIN$_3$ RECEPTOR AGONIST SR57227A ON MESO-CORTICOLIMBIC DOPAMINE TARGET NEURONS IN RATS. Jian Yu Zhang*, Bryan Horan and Rex Y. Wang. Dept. of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, Stony Brook, NY 11794-8790.

We have previously shown that 5-HT$_3$-like receptors in the medial prefrontal cortical (mPFC) have a permissive role in regulating or gating the inhibitory action of dopamine (DA). In the present study, we examined further the effect by which a potent and selective 5-HT$_3$ receptor agonist SR 57227A modulates the depressant action of DA on cells in the mPFC, hippocampus and nucleus accumbens (NAc), using the techniques of single cell recording and iontophoresis. We have previously demonstrated that SR 57227A produced a current-dependent depression of the firing of hippocampal CA1 pyramidal cells and this effect was blocked by the selective 5-HT$_3$ but not other receptor antagonists. The iontophoretic application of DA (5-80 nA) produced a current-dependent depression of both spontaneously active and glutamate-evoked firing of cells in the mPFC, NAc and hipocampus. When SR 57227A was iontophoresed concurrently with DA at a subthreshold current, it significantly potentiated the inhibitory action of DA but not GABA. The potentiation was blocked by the selective 5-HT$_3$ receptor antagonist BRL 46470A. Our results support and extend the previous finding that 5-HT$_3$ receptors play an important role in modulating the DA's action. Since the 5-HT$_3$ receptors are primarily localized in the mesocorticolimbic structures, it is possible that the simultaneous blockade of DA and 5-HT$_3$-like receptors by clozapine may partially account for its preferential interaction with the mesocorticolimbic DA system. In addition, this may also contribute to clozapine's higher therapeutic efficacy (Supported by MH-41440).

808.7

ABILITY OF TRICHLOROETHANOL TO MODIFY 5-HYDROXYTRYPTAMINE$_3$ RECEPTOR MEDIATED DEPOLARISATION OF THE ISOLATED RAT VAGUS NERVE. KR Bentley*, KD Johnston, RL Stowe and NM Barnes. Dept of Pharmacology, Medical School, University of Birmingham, Birmingham B15 2TT UK.

In the present study we assess the ability of trichloroethanol (TCE) to modulate the function of the 5-HT$_3$ receptor expressed in rat vagus nerve measured by extracellular recording (Blake JF et al. Br J Pharmacol 95; 291-299, 1988).

5-HT (10 nM-30 µM) induced depolarizations of the rat vagus nerve (EC_{50} = 0.97 ± 0.13 µM, (mean ± SEM, n = 5)) that were antagonized by the selective 5HT$_3$ receptor antagonist ondansetron (20 nM; pK_B = 8.9).

TCE (5 mM) significantly increased the potency and maximal response of 5-HT to depolarize the vagus nerve (5-HT; EC_{50} = 0.5 ± 0.12 µM, mean ± SEM, n = 5). TCE (0.1-10 mM) maximally increased the response to a submaximal concentration of 5-HT (0.3 µM), TCE pEC_{50} = 2.55 ± 0.13, (mean ± SEM, n = 3). 5-HT (0.3 µM)-induced depolarisations in the presence of TCE at concentrations above 10 mM were submaximal.

The selective 5-HT$_3$ receptor agonist, phenylbiguanide (PBG) induced depolarizations of the isolated rat vagus nerve with near the same potency as 5-HT (EC_{50} = 1.49 ± 0.33 µM, mean ± SEM, n = 3) and these were also potentiated by TCE (5 mM) (PBG; EC_{50} = 0.28 ± 0.07 µM, maximal response relative to PBG alone = 124 ± 20% mean ± SEM, n = 3, $p < 0.05$, Students paired t-test). Similarly, TCE (5 mM) significantly increased the size of the depolarisation induced by the low intrinsic activity partial agonist quipazine (300 nM) whereas, even in the presence of TCE (5 mM), we failed to detect a response on application of (S)-zacopride (300 nM). The present study has identified that TCE enhances the potency and the maximal response of 5-HT$_3$ receptor agonists to depolarize the isolated rat vagus nerve.

Supported by the MRC.

808.8

DISTRIBUTION OF 5-HYDROXYTRYPTAMINE3 RECEPTOR EXPRESSION IN THE HUMAN FOREBRAIN

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The distribution of 5-hydroxytryptamine3 (5-HT3) receptor expression in the human forebrain was assessed using quantitative receptor autoradiography with [^{3}H]-(S)-zacopride and Northern blot analysis to detect 5-HT3 receptor-A/As receptor subunit mRNA levels using [^{32}P]riboprobes generated from 5-HT3-As cDNA (Hope et al, Eur J Pharmacol 245, 187-192, 1993).

5-HT3 receptor expression was differentially distributed with highest levels in the hippocampus (autoradiographic studies revealed that within heterogeneous structure, the levels of expression (fmol/mg tissue equivalent; Amersham; mean±SEM, n = 3-7) were highest in the dentate granule cell layer (16±2), dentate molecular cell layer (8.1±1.2)) and the extrapyramidal system (autoradiographic studies; caudate nucleus (4.3±0.8), putamen (4.3±0.8), substantia nigra (2.3±0.6), mean±SEM, n = 3-7). No 5-HT3 receptor expression was detected in the cerebellar cortex.

In the present studies, 5-HT3 receptor expression in the human forebrain was highest in the hippocampus and striatum (caudate nucleus and putamen). Ongoing in situ hybridisation studies may reveal which neurones express the 5-HT3 receptor.

We are grateful to Drs A.G. Hope, J.A. Peters and Prof. J.J Lambert for the gift of 5-HT3-As cDNA. Supported by the MRC.

808.9

NEUROCHEMICAL COMPOSITION OF NEURONS EXPRESSING THE 5-HT$_3$ RECEPTOR. M. Morales*, E. Battenberg and F. E. Bloom. The Scripps Research Institute, Neuropharmacology. 10666 N. Torrey Pines Rd., La Jolla CA 92037.

The type 3 serotonin receptor (5-HT$_3$R) is a ligand-gated ion channel whose presence in the central nervous system has been established by radioligand binding and *in situ* hybridization analysis.

In a previous study (Soc. Neurosci. Abstr., 20:1156), we combined *in situ* hybridization and immunocytochemistry techniques to identify neurotransmitters present in 5-HT$_3$R expressing cells. We detected a substantial number of neurons expressing both 5-HT$_3$R and gamma-amino butyric acid (GABA) throughout the brain. To further characterize the 5-HT$_3$R/GABA expressing neurons we investigated if the neuropeptides somatostatin (SS) or cholecystokinin (CCK) were present in 5-HT$_3$R expressing neurons. While SS was not found in 5-HT$_3$R expressing neurons, co-expression was found for 5-HT$_3$R and CCK. As subpopulations of GABAergic cells might contain different Ca^{2+}-binding proteins (calbindin, calretinin or parvalbumin) we sought to determine if any of these proteins were present in 5-HT$_3$R expressing cells. We found that while calbindin did not co-localize with 5-HT$_3$R transcripts, limited co-localization was found for calretinin, whereas, parvalbumin (PV) was often found in cells expressing 5-HT$_3$R.

We conclude that the inhibitory 5-HT$_3$R/GABA interneurons may be basket cells that coexist with parvalbumin or CCK. Our data on the presence of 5-HT$_3$R/CCK-containing neurons in cortex and hippocampus provide anatomical evidence that activation of local 5-HT$_3$R/CCK expressing cells might participate in the release of CCK in cortex and the hippocampus.

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SEROTONIN

809.1

SELECTIVE MODULATION OF TRYPTOPHAN HYDROXYLASE AND TYROSINE HYDROXYLASE EXPRESSIONS BY S-20342 IN THE RAT BRAIN. S. Raison[1], D. Weissmann[1], M.C. Rettori*[2], H.-P. Husson[3], P. Renard[2], B. Guardiola-Lemaitre[2] and J.-F. Puiol[1]. [1]CNRS-UCB, UMR 105, Rue G. Paradin, 69372 Lyon; [2]I.R.I.SERVIER, 6 Pl. des Pléiades, 92415 Courbevoie; [3]Laboratoire de Chimie Thérapeutique, 4 ave de l'Observatoire, 75006 Paris, FRANCE.

S-20342 (-N-[(1R,4R,9aS)-4-phenyl octahydropyrido [2,1-c] [1,4] oxazin-1-yl] 3,4,5-trimethoxybenzamide) is a new chemical entity with an unexpected neurochemical and behavioral profile: although devoid of any affinity for 5HT and DA receptor subtypes, it produced in animal models anxiolytic-like and antipsychotic-like effects[(1)]. In this study, tryptophan hydroxylase (TpOH) protein was quantified by dot blot analysis in steady-state conditions and 12 hours after a single injection of *p*-chlorophenylalanine (ip; 300 mg/kg), to estimate the apparent disappearance rate of TpOH protein, in control and S-20342 treated rats (ip; 30 mg/kg). This quantitative analysis was performed in the mesencephalic serotoninergic cell body groups, the raphe dorsalis (RD) and centralis (RC) nuclei as well as in their respective terminal areas, the neostriatum and the hippocampus. The timecourse effect, performed 1, 2, 3, 4 and 6 days after S-20342 injection showed a significant increase in the steady-state content of TpOH in the RD at day 4. The turnover rate of TpOH was highly enhanced in both RD and RC, by 18% and 21% respectively, at day 1, and by 57% and 45% at day 4. Furthermore, a highly significant increase in TpOH mRNA content was quantified by *in situ* hybridization, 12 hours after S-20342 injection, in the RD. A slight increase in TpOH protein content was observed in the hippocampus but no modification was measured in the neostriatum. Interestingly, tyrosine hydroxylase protein was significantly increased at day 3 in the ventral tegmental area but neither in substantia nigra nor in locus coeruleus. These results suggest that S-20342 would potentially induce TpOH protein, which is of particular interest to further study the regulation of TpOH expression.

(1) M.C. RETTORI et al. - XIII ISMC Congress - Paris, France, 19-23 Sept. 1994

809.2

EVIDENCE FOR PRESYNAPTIC COMPENSATORY CHANGES IN SEROTONERGIC NEURONS FOLLOWING DESTRUCTION WITH 5, 7-DIHYDROXYTRYPTAMINE. A.C. DeVries, F.S. Hall, M. Linnoila* and A. Pert. NIMH and NIAAA, Bethesda, MD 20892.

Following selective neurotoxin induced lesions of the nigro-striatal dopamine (DA) system, it has been found that presynaptic compensatory changes take place in the remaining populations of DA neurons sufficient to normalize the extracellular concentrations of striatal DA. The purpose of the present study was to determine whether the serotonergic (5-HT) system has similar characteristics following neuronal destruction. Rats implanted with microdialysis guide cannulae in the striatum received intracerebroventricular injections of either 0, 50, 100, 150, or 200 µg of 5,7-dihydroxytryptamine (5,7-DHT, a selective 5-HT neurotoxin). Two to three weeks later the animals were tested behaviorally, and then had a CMA-11 microdialysis probe introduced into the striatum. Twenty four hours later microdialysis samples were collected at 30 min intervals and analyzed for 5-HT content with standard HPLC-EC procedures using a microbore column. Lesions produced by the highest concentrations of 5,7-DHT (200 µg) proved to be anxiolytic while lesions produced by lower concentrations of 5,7-DHT (150 and 100 µg) were anxiogenic as assessed with an elevated plus-maze. Low and medium doses of 5,7-DHT failed to alter significantly extracellular levels of 5-HT in the striatum while reducing tissue levels across a variety of terminal and perikaryal regions. Only the highest dose of 5,7-DHT was able to decrease striatal extracellular 5-HT, which was also accompanied by sustantial striatal depletions of tissue 5-HT. It appears that presynaptic compensatory changes take place in 5-HT neurons following lesioning similar to those seen in the dopaminergic systems.

809.3

AN ANTISENSE OLIGONUCLEOTIDE TO TRYPTOPHAN HYDROXYLASE HAS DIFFERING EFFECTS ON INDICES OF SEROTONERGIC FUNCTION. F. S. Hall*, A. Pert, D.A. Nielsen, M. Linnoila Laboratory of Clinical Studies/DICBR, NIAAA and Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

The effects of an antisense phosphothioate oligonucleotide aimed at position 123 of rat tryptophan hydroxylase mRNA (5' TGT CTT CAA TCA TGG 3') was compared to a random sequence (5' TCG AAT CGT TAA TCG 3'). The compounds were administered directly into the median raphe nucleus via an injector inserted into an indwelling guide cannula (AP -7.7, ML +0.0, DV -7.5) in male Sprague Dawley rats. Subjects received 3 injections of 1 nmol oligonucleotide in 1ul of saline at 12 hour intervals. Twenty-four hours after the last injection the subjects were decapitated and discrete brain regions were dissected from the tissue: frontal cortex, ventral striatum, dorsal striatum, hypothalamus, hippocampus, dorsal raphe nucleus, and median raphe nucleus. Tissue samples were subsequently analyzed for serotonin, dopamine, norepinephrine and 5-hydroxytryptophan content using HPLC-EC. Prior to sacrifice, food consumption was found to be increased in antisense-treated subjects and open-arm entries in the elevated plus maze were decreased compared to control subjects. In these subjects tissue serotonin, 5-hydroxytryptophan, dopamine and norepinephrine content were unaltered. Investigations are underway to examine other neurochemical indices of serotonin function which might account for the observed behavioral differences in antisense-treated subjects.

809.4

Effect of Estrogen (E) and Progesterone (P) on the Expression of Tryptophan Hydroxylase (TPH) mRNA in the Raphe Nucleus of Non-human Primates. M. Pecins-Thompson, N.A. Brown, C.L. Bethea*, Division of Reproductive Sciences, Oregon Regional Primate Research Center, Beaverton, OR 97006

P increases prolactin secretion in E-primed female monkeys through a neural mechanism and serotonin (5HT) is a stimulatory transmitter for prolactin. This laboratory has shown that E induces progestin receptors in 5HT neurons. To determine whether E or P alters the function of 5HT neurons, the expression of mRNA for tryptophan hydroxylase (TPH) was examined in ovariectomized (ovx)-control, E treated (28 days) and E+P treated monkeys (14 days E + 14 days E+P) using *in situ* hybridization and a 248 bp TPH probe generated with RT-PCR (n=3 animals/group). Perfusion fixed midbrain sections (10µ) were hybridized at 40°C with ^{35}S antisense cRNA directed against the 5' substrate binding domain of monkey TPH. Adjacent sections were immunostained for 5HT to confirm the location of the dorsal raphe (DR). After a final wash in 0.1X SSC at 50°C, sections were opposed to tritum sensitive film for 14 days. Densitometric analysis with graylevel thresholding was performed at 3 or 4 levels of the DR. The number of pixels exceeding background in defined areas was obtained (*pixel number*) and normalized by the total number of pixels counted (*pixel fraction*). There was a 10 fold increase in TPH mRNA signal represented by both pixel number and pixel fraction in E treated macaques compared to ovx controls ($p < 0.05$). Supplemental P treatment reduced TPH mRNA expression from E levels in 2/3 animals but the average TPH mRNA level in E+P treated animals remained 4 fold higher than in ovx controls. These data indicate that E induces TPH gene expression in non-human primates. The effect of E+P on TPH mRNA was variable with the average level falling between the ovx group and the E treated group. Supported by HD17269, HD18185, DK9098, RR00163

809.5

EFFECTS OF ALTERED L-TRYPTOPHAN AVAILABILITY ON THE BRAIN RELEASE AND TURNOVER OF SEROTONIN IN PORTACAVAL SHUNTED RATS AS ASSESSED BY IN VIVO MICRODIALYSIS. P.B.F. Bergqvist*, S. Hjorth, G. Apelqvist, and F. Bengtsson, Depts of Clin Pharmacol, Lund Univ Hosp, 221 85 Lund, and Pharmacol, Univ of Gothenburg, 413 90 Gothenburg, Sweden.

A large body of evidence suggests that the neuropsychiatric syndrome seen in liver failure, hepatic encephalopathy (HE), is associated with an increased brain turnover (t.o.) of serotonin (5-HT). However, in a recent study we showed an unaltered neuronal release of 5-HT in chronic HE as reflected by unchanged dialysate 5-HT levels in the brain of portacaval shunted (PCS) rat. In the present study we studied the frontal neocortical extracellular levels of 5-HT and its main metabolite 5-HIAA at basal HE conditions and after a provocation of the 5-HT system by systemic administration of the precursor amino acid L-tryptophan (L-TRP; 1 loading dose of 280 mg/kg i.p. followed 3 hours later by 5 consecutive iterations of 50 mg/kg during 5 hours). Brain 5-HT t.o. was also determined by studying brain extracellular accumulation of 5-HIAA following systemic administration of probenecid (200 mg/kg i.p.), an inhibitor of the brain outtransport mechanism of 5-HIAA. While the basal extracellular 5-HT levels were unaffected by the PCS procedure the 5-HIAA levels were elevated ($p<0.05$) in the PCS rats compared with controls confirming the contention that increased brain 5-HT t.o. in HE is not associated with an increased neuronal release of 5-HT. Following the L-TRP administrations the 5-HT levels did not change either in PCS or control rats. The 5-HIAA levels, however, increased both in the PCS rats but especially in the controls. This finding may indicate that the 5-HT t.o. in the control rats are more susceptible to increased L-TRP availability. The probenecid treatment resulted in a similar 5-HIAA accumultaion in PCS and control rats. This similar time-course for the 5-HIAA accumulation might indicate that the increased extracellular 5-HIAA levels and, hence, the increased 5-HT t.o., is a phenomenon partly due to inhibition of the brain 5-HIAA outtransport.

809.6

SEASONAL VARIATION IN NEUROENDOCRINE AND MOOD RESPONSE TO L-TRYPTOPHAN INFUSION IN DEPRESSED PATIENTS AND HEALTHY SUBJECTS. A. Cappiello*, R. T. Malison, C. J. McDougle, S. Vegso, D. S. Charney, G. R. Heninger, L. H. Price. Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06519.

Recent reports suggest that seasonal variation of mood disorders might be related to alteration in rhythmicity of serotonin [5-HT] function. In this study we examined seasonal effects on the neuroendocrine and mood responses to i.v. infusion of the 5-HT precursor L-tryptophan (L-TRP) in depressed patients and healthy subjects. Methods: 126 drug-free patients with DSM-III-R major depression and 58 healthy comparison subjects participated. After an overnight fast, subject received an i.v. infusion of L-TRP 7 g. Blood was obtained for determination of serum prolactin (PRL), growth hormone (GH), and tryptophan levels. Visual analogue scales were used to assess mood. Results: Cosinor analysis revealed seasonal variation in peak change (Δ) PRL in the combined depressed patients ($p<.02$) and in unipolar ($p<.04$), nonmelancholic ($p<.003$), and nonpsychotic ($p<.007$) subgroups, with winter acrophases and summer troughs; healthy subjects showed no seasonality. Peak Δ GH showed seasonal variation in healthy subjects (April acrophase and February trough, $p<.01$), but not in depressed patients. Baseline tryptophan levels demonstrated summer peaks and winter troughs in the combined depressed group ($p<.01$) and in unipolar ($p<.009$), nonmelancholic ($p<.02$), and nonpsychotic ($p<.03$) subgroups. A negative correlation was found between peak Δ PRL and tryptophan levels in combined depressed ($p<.02$) and unipolar patients ($p<.04$). Baseline GH levels also manifested seasonal variation in the combined depressed ($p<.01$) and in bipolar ($p<.01$), melancholic ($p<.001$), nonpsychotic ($p<.04$), and psychotic ($p<.007$) patients. Conclusions: Our data are consistent with previous evidence that central 5-HT function is abnormal in depressed patients and further suggest a seasonal variability of such abnormalities that is absent in healthy subjects. Seasonal patterns of 5-HT function confined to specific diagnostic subgroups suggest that the pathophysiologies of clinically discrete mood disorder subgroups may be heterogeneous.

809.7

THE STIMULATION OF FORWARD LOCOMOTION ELICITED BY MK-801 IS ABOLISHED BY LOW DOSES OF THE 5-HT_{2A} ANTAGONIST MDL 100,907 P. Martin*, N. Waters, S. Holm, A. Carlsson and M. L. Carlsson.

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1. In a previously published report (P. Martin *et al.*, J Neural Transm [Gen Sect] 1994; 95 :113-121), the dopamine D-1-antagonist SCH 23390, was more efficient than the D-2-antagonist raclopride, in counteracting the hyperactivity induced by the non-competitive NMDA-receptor antagonist MK-801. However, SCH 23390, besides its D-1 affinity, has been shown to have affinity for 5-HT_2-receptors. 2. Here, 3 compounds selective for the D-1, D-2 and 5-HT_{2a}-receptors, respectively, were tested concerning their effect on normal locomotion and MK-801-induced (0.3 mg/kg i.p.) hyperlocomotion in mice. 3. The D-1 antagonist SDZ 219-958 (0.017-0.15-1.35 mg/kg i.p.) dose-dependently counteracted the MK-801-induced locomotor stimulation. However, this was also the case for the 5-HT_{2a}-receptor antagonist MDL 100,907 (0.001-0.01-0.1 mg/kg i.p.), which even abolished (0.1 mg/kg) the MK-801-induced hyperlocomotion, with relatively less influence on locomotion in normal animals. 4. Finally, the effect in normal and MK-801 pretreated mice, of the concomitant administration of either of the D-1 or the D-2 antagonist (raclopride) with the 5-HT_{2a}-antagonist, was investigated. Protocols based on orthogonal design matrices were used. Data was subjected to multiple regression and the results presented as 3-dimensional response surface graphs. The main finding of these experiments was that regression coefficients for MDL 100,907, being insignificant in normal mice, became larger and highly significant in mice pretreated with MK-801. 5. In conclusion, low doses of the 5-HT_{2a}-receptor antagonist MDL 100,907 given alone or during simultaneous blockade of D-1 or D-2 receptors, have a strong inhibitory effect on MK-801-induced hyperlocomotion in mice. The equally strong inhibitory effect, in this respect, reported earlier with SCH 23390, is not explained solely by its D-1 antagonistic properties, and might partly be due to its affinity for 5-HT_2-receptors.

809.8

ROLE OF NITRIC OXIDE (NO) IN PENILE ERECTION AND YAWNING INDUCED BY 5-HT_{1C} RECEPTOR AGONISTS. A. Argiolas* & M.R. Melis. Bernard. B. Brodie Dept. Neurosci., Cagliari Univ., 09124 Cagliari, Italy.

The effect of N^G-nitro-L-arginine methyl ester (NAME) and N^G-monomethyl-L-arginine (NMMA), two inhibitors of NO synthase, given into a lateral ventricle (i.c.v.) on penile erection and yawning induced by 1-(3-chlorophenyl)-piperazine (m-CPP) and N-(3-trifluoromethylphenyl)-piperazine (TFMPP) two selective 5HT_{1C} receptor agonists, was studied in male rats. Both NO synthase inhibitors (50-500 μg) prevented dose-dependently the behavioral responses induced by m-CPP (0.5 mg/kg s.c.) or by TFMPP (1 mg/kg s.c.), NAME was about 4-5 times more potent than NMMA while D-NMMA, which does not inhibit NO synthase, was ineffective. The inhibitory effect of NAME on m-CPP and TFMPP responses was prevented by the co-administration of L-arginine (1 mg i.c.v.). m-CPP- and TFMPP-induced penile erection and yawning was prevented also by the i.c.v. administration of LY 83583 (50-200 μg) or methylene blue (50-400 μg), two inhibitors of guanylate cyclase but not by reduced hemo-globin (50-400 μg), a NO scavenger. The results suggest that central NO is involved in 5-HT_{1C} receptor agonist-induced penile erection and yawning.

809.9

THE SEROTONIN 5-$HT_{2A/2C}$ SELECTIVE AGONIST, DOI ELICITS HEAD TWITCH FOLLOWING DIRECT INJECTION INTO THE MEDIAL PREFRONTAL CORTEX OF RATS. David L. Willins* and Herbert Y. Meltzer, Laboratory of Biological Psychiatry, Case Western Reserve University, Cleveland, Ohio 44106

The systemic administration of selective serotonin-2 (5-HT_2) receptor agonists produces a characteristic head twitch response (HTR) in rodents. The selective 5-$HT_{2A/2C}$ agonist, DOI produces a HTR following bilateral injection into the medial prefrontal cortex (mPFCx) of male, Sprague-Dawley rats. This response was dose-dependant for doses ranging from 0 to 57 nmoles/0.5 μl/side, and was reversed by pretreatment with ketanserin (2.5 mg/kg, i.p.). In addition, pretreatment of rats with either clozapine (1 mg/kg, i.p.), amperozide (5 mg/kg, i.p.), remoxipride (10 mg/kg, s.c.) or SCH23390 (50 μg/kg, s.c.) also inhibited DOI-induced head twitch. Intra-cortical administration of MK-212, a serotonin agonist which has 100-fold greater selectivity for the 5-HT_{2C} receptor than the 5-HT_{2A} receptor, did not produce a HTR when administered at a dose of 34 nmoles/0.5 μl/side. Chronic treatment of rats with antipsychotic drugs has been shown to alter both serotonergic and dopaminergic receptors. Twenty-one day treatment of rats with either clozapine (20 mg/kg/d, p.o.) or haloperidol (0.5 mg/kg/d, p.o.) inhibited the HTR to DOI. Finally, pretreatment of rats with baclofen, a $GABA_B$ receptor agonist, was shown to produce a bi-phasic alteration of the HTR to DOI. While a higher dose of baclofen (3 mg/kg, sc) produced a marked inhibition of DOI-induced head twitch, a low dose (0.6 mg/kg, sc) potentiated this response. These data suggest that activation of 5$HT2_A$ receptors in the mPFCx stimulates a HTR which may involve dopaminergic as well as GABAergic mechanisms. (Supported by National Alliance for Research on Schizophrenia and Depression)

809.10

VOLTAMMETRIC MEASUREMENT OF SEROTONIN IN THE SUBSTANTIA NIGRA PARS RETICULATA OF FREELY MOVING RATS. J.L. Góngora-Alfaro*, S. Hernández, F. Heredia, J.L. Bata, G. Arankowsky, J. Aceves and D. Martínez-Fong, Centro de Investig. Regionales, Universidad Autónoma de Yucatán, México, 97000.

The substantia nigra pars reticulata (SNr) receives serotonergic innervation from the raphe nuclei. Using differential pulse voltammetry with carbon fiber microelectrodes (CFM) we have measured the extracellular concentration of serotonin (5-HT) in the SNr of freely moving rats (n=5). Thirty six hours after implanting the CFM, an oxidation peak was measured at +277 mV. Allopurinol (20 mg/kg, i.p.) reduced the peak height to 55 ± 4%, indicating the contribution of uric acid (15.5 ± 4.4 μM) to the oxidation peak. Pargyline (40 mg/kg, i.p.) caused a further reduction of the peak height to 32 ± 3%, indicating that 5-HIAA (1 ± 0.1 μM) also contributes to the peak. The remaining peak was considered to be 5-HT because it appeared at +307 ± 6 mV and was increased by the 5-HT reuptake blocker duloxetine. The basal concentration of 5-HT (45.6 ± 10.8 nM) could be an overestimation due to the inhibition of 5-HT metabolism by pargyline. It is concluded that 5-HT is released in the SNr. (Supported by grant 1831-M9211 from CONACyT, México.)

809.11

Neurochemical and behavioral correlates in post-hypoxic myoclonus: an *in vivo* microdialysis study. A.G. Kanthasamy*, T.Q. Vu, S.P. Jaw and D.D. Truong, Parkinson & Movement Disorders Laboratory, Dept. of Neurology, Univ. of California, Irvine, CA 92717.

To evaluate the neurochemical dynamics of serotonergic and dopaminergic systems in post-hypoxic myoclonus, the extracelluar release of serotonin (5-HT), dopamine (DA) and their metabolites were determined using *in vivo* microdialysis. Basal and stimulated release of 5-HT, 5-HIAA, DA and DOPAC were monitored in the prefrontal cortex of a stimulus-sensitive myoclonus model. KCl (100 mM) or NMDA (500 μM) were locally infused to evoke the release of neurotransmitters. Basal levels of these neurotransmitters were unaffected among the three groups (control, post-hypoxic, post hypoxic-recovered rats). A significant reduction ($p < 0.01$) in KCl- and NMDA-stimulated release of 5-HT and DA was observed between control and post-hypoxic rats. The depolarization-induced reduction in 5HIAA was markedly elevated in post-hypoxic rat as compared to controls. These neurochemical changes returned close to normal levels in post hypoxic-recovered rats. A linear relationship was obtained between neurochemical alterations and behavioral quantitation of myoclonus. These data suggest that a synergistic hypofunctioning of stimulus-induced serotonergic and dopaminergic terminals in mesocortical regions may contribute to the behavioral expression of post-hypoxic myoclonus. (Supported by Myoclonus Research Foundation).

809.12

SEROTONERGIC MODULATION OF AUDITORY EVOKED POTENTIALS IN BEHAVING CATS. G. Juckel, M. Mölnar, U. Hegerl, V. Csepe, G. Karmos (SPON: European Neuroscience Association). Depts. Psychophysiology, Freie Universität Berlin (Germany) and Academy of Sciences Budapest (Hungary).

Several findings suggest that the stimulus intensity dependence of auditory evoked potentials is modulated by the brain serotonin system Epidural recordings over the primary and secondary auditory cortex were conducted in chronically implanted cats under drugs influencing the serotonergic system administered either i.v. or by local injection into the dorsal raphe. The intensity dependence was increased after blocking serotonergic activity presynaptically and decreased after stimulating this activity. Postsynaptically, modifications of 5-HT_{1a} and 5-HT_2 receptors, but not of 5-HT_{1c} receptors, changed the intensity dependence. The serotonergic modulation of the intensity dependence was stronger in the primary than in the secondary auditory cortex, possibly corresponding to the known different serotonergic innervation of the two areas. The serotonergic system seems to modulate the initial stage of auditory processing, since only the first component of the evoked potentials was affected by the serotonergic interventions.

809.13

EFFECT OF REPEATED EXPOSURE TO FORCED SWIMMING STRESS ON EXTRACELLULAR LEVELS OF 5-HT IN THE RAT. L. Kirby* and I. Lucki. Departments of Psychiatry and Pharmacology, Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Previous work using *in vivo* microdialysis demonstrated that forced swimming produces regionally-selective changes in extracellular 5-hydroxytryptamine (5-HT) in rats (Kirby et al., Brain Res., 1995, in press). Swimming produced increases of 5-HT in striatum, decreases of 5-HT in amygdala and lateral septum, and did not change 5-HT in frontal cortex and hippocampus. This study examined these neurochemical changes after repeated exposure to the swim stress. Cannulae were implanted under surgical anesthesia into either striatum or lateral septum. One week later, dialysis probes were lowered through the cannulae and dialysis samples collected before, during, and after a 15 min swim exposure. This dialysis procedure and swim exposure was repeated the following day. Dialysate content of 5-HT and 5-hydroxyindole-acetic acid (5-HIAA) were measured by HPLC. On the first test day, swim produced an elevation of extracellular 5-HT by 60% over baseline in striatum and a reduction to 40% below baseline in lateral septum. On the second day, swim had no effect on 5-HT in either brain region. On both days, however, swim produced decreases in 5-HIAA in both regions to 40-50% below baseline. These results suggest that there is an adaptation of the regionally-specific effects of forced swimming on extracellular 5-HT, but not of 5-HIAA, over repeated exposure to the stress. *Supported by USPHS grants MH 17168, MH36262, and MH 48125*

809.14

EFFECT OF FORCED SWIMMING STRESS ON EXTRACELLULAR LEVELS OF 5-HT IN THE DORSAL RAPHE NUCLEUS OF THE RAT. J. M. Chou*, L. G. Kirby, and I. Lucki. Depts. of Psychiatry and Pharmacology, Inst. of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

The effect of forced swimming for 30 minutes on extracellular levels of 5-hydroxytryptamine (5-HT) and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) were examined in the dorsal raphe nucleus, a region containing 5-HT cell bodies. Extracellular levels of 5-HT and 5-HIAA were measured using *in vivo* microdialysis with electrochemical detection with 10 min resolution. A dialysis probe was implanted into the dorsal raphe nucleus under surgical anesthesia on the day prior to the study. The next day, dialysate samples were collected for 70 min to establish baseline. Forced swimming initally produced a 40% decrease in raphe extracellular concentrations of 5-HT and 5-HIAA. 5-HT concentration remained supressed for approximately 2 hours following the swim. 5-HIAA concentration remained reduced for 30 min, but rebounded to increase 20% above baseline 2 hours after the swim. This indicates that 5-HT metabolism in the dorsal raphe nucleus was likely increased while 5-HT release was reduced by the forced swim. Previous work in our laboratory has shown that forced swimming produced a regionally- selective pattern of changes in extracellular 5-HT (Kirby et al., Brain Res., 1995, *in press*). Since forced swimming is used as a screen for antidepressant drug effects, it is possible that regional changes in levels of 5-HT may play a selective role in mediating the behavioral effects of antidepressant drug treatments. *Supported by USPHS grants MH 17168, MH36262, and MH 48125*

809.15

PERIPHERAL AND CENTRAL INDICES OF SEROTONIN FUNCTION AND IMPULSIVITY. C. Reist*, D. Helmeste, L. Albers, S.W. Tang. UC-Irvine, Psychiatry Service, VA Medical Center, Long Beach, CA, 90822.

A large body of literature exists implicating serotonin in psychiatric and behavioral disorders. There is evidence for its role in suicide, aggression and other behaviors characterized by impulsivity. Efforts continue to refine serotonergic challenge paradigms and to develop new tools to examine this system at the molecular level. In the present study hormonal response to oral paroxetine was examined for use as a serotonergic challenge agent in a group of healthy subjects. Platelet $5HT_{2A}$ mediated intracellular calcium response to serotonin was also measured to assess signal transduction. Paroxetine elicited a robust cortisol response which was directly correlated with the magnitude of platelet calcium response. Both of these measures were inversely correlated with the trait of impulsivity as measured by the Barratt Impulsivity Scale. These results suggest that paroxetine has utility in studying serotonergic systems.

810.1

ATP-INDUCED OSCILLATIONS OF CYTOSOLIC CA2+ ACTIVITY IN CULTURED ASTROCYTES AND IN GLIOMA CELLS. G. Reetz*. Institute of Neurobiochemistry, University of Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany.

Continuous stimulation with ATP induced $[Ca^{2+}]_i$ oscillations (1-2/min) in cultured astrocytes from newborn rats and in rat glioma cells (C6-4-2). The initial $[Ca^{2+}]_i$ response to ATP resulted from $InsP_3$-induced Ca^{2+} release, whereas the subsequent oscillations were dependent on both $InsP_3$-sensitive Ca^{2+} stores and Ca^{2+} influx. Depolarization by 50 mM $[K^+]_{ex}$ resulted in a transient Ca^{2+} response in astrocytes, presumably due to activation of voltage-dependent Ca^{2+} channels. In C6-4-2 glioma cells, $[Ca^{2+}]_i$ oscillations were also induced by bradykinin. Simultaneous recording of membrane potential showed that the oscillations of $[Ca^{2+}]_i$ and of membrane potential in glioma cells were synchronous. The oscillations were affected by the K^+ equilibrium potential and by blocking $K^+(Ca^{2+})$ channels, indicating a potentiation of Ca^{2+} influx by membrane hyperpolarization. Whole-cell patch-clamp experiments indicate a conductance for Na^+ and for Ca^{2+}.

The oscillations were also influenced by hypotonic and by hypertonic medium in glioma cells as well as in astrocytes. We conclude that in glial cells there is a feedback regulation between cell volume and $[Ca^{2+}]_i$. The experiments indicate a possible physiological function of Ca^{2+} oscillations in volume regulation of glial cells.

810.2

A DETERGENT, CYTOCLEAN, HAS SELECTIVE ACTIONS ON LIGAND-GATED ION CHANNELS EXPRESSED IN XENOPUS OOCYTES. T.K. Machu*[1], S.J. Mihic[2], X.-P. Qian[1], and J.E. Dildy-Mayfield[2] [1]Dept. of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430 [2]Dept. of Pharmacology, University of Colorado Health Sciences Center and VA Medical Center, Denver, CO 80262

Cytoclean was observed to have selective actions on ligand-gated ion channels. Responses of α1 and α2 glycine receptors expressed in Xenopus oocytes were 3 to 5 fold higher with solutions prepared in glassware that had been washed with Cytoclean than with solutions prepared in glassware that had been extensively washed with ethanol and Nanopure water. Cytoclean (0.001%-0.01%) potentiated 50 μM glycine responses in oocytes expressing α2 glycine receptors by 23 ± 7% to 342 ± 43%. Cytoclean is composed of 4 reagents dissolved in water, and these reagents were tested individually for their abilities to modulate responses of oocytes expressing α2 glycine receptors. Tetrapotassium phosphate, sodium xylene sulfonate, and Triton X-100 (1-10 fold of their respective concentrations in Cytoclean) minimally potentiated 50 μM glycine mediated currents. Approximately 10% of Cytoclean is composed of Biosoft D62, which at concentrations of 0.00005%-0.001%, potentiated 50 μM glycine responses by 13±1% to 474±50%. Biosoft D62 is composed of ethanol (29%) and linear alkylbenzene sulfonate (>95% C12 chain). Dodecanol is used in the synthesis of Biosoft D62 and represents no more than 1.5% of this reagent. Neither the concentration of ethanol (~5 mM) nor dodecanol (~ 4 μM) that is present in the highest concentration of Biosoft D62 tested had any effect on 50 μM glycine mediated currents, suggesting that the reagent responsible for the potentiation of glycine receptor function is linear alkylbenzenesulfonate. The effects of Cytoclean were examined on 5-HT_3, $GABA_A$, and kainate/AMPA receptors expressed in Xenopus oocytes. Cytoclean had no effect on 5-HT_3 nor GluR6 receptor function, but Cytoclean (0.005% and 0.01%) inhibited GluR3 mediated currents by ~21% and 41%, respectively. Enhancement of $GABA_A$ receptor function ranged from 21±2.3% to 458±142% with Cytoclean (0.0001% to 0.01%), respectively. These results suggest that trace amounts of Cytoclean adhering to glassware may confound experimental results.

810.3

EXAMINATION OF BOVINE APO-OPSIN AS A MODEL OF LIGAND BINDING RECEPTORS. W.A. Clark*, J.C. Gutierrez, and J.K. Northup. Lab of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892-4090.

Rhodopsin and the associated G-protein transducin have been utilized by numerous investigators to examine the mechanism of receptor-G-protein interaction. We have extended this work using apo-opsin to serve as a model for ligand binding seven transmembrane receptors. Bovine rhodopsin-containing rod outer segment disc membranes were depleted of chromophore using hydroxylamine at basic pH. In an established *in vitro* transducin activation assay (Fawzi et al., JBC 266:12194, 1991), the resultant apo-opsin demonstrated ~50-fold lower activity than rhodopsin. Reconstitution of this opsin with 9-*cis*- or 13-*cis*-retinal in the dark results in a significant inhibition of opsin activity below basal. Upon exposure of this "inverse agonist"-bound state of opsin to light, full rhodopsin activity is achieved. Furthermore, apo-opsin responds to all *trans*-retinal in a dose dependent manner to form a reactivated rhodopsin species indistinguishable from native rhodopsin in the transducin activation assay. Data are presented demonstrating the effects of transducin in modulating the apparent affinity of opsin for all *trans*-retinal. These studies utilizing opsin provide novel insights into the functioning of this class of receptors.

810.4

CAFFEINE-INDUCED FOS PROTEIN EXPRESSION IN THE RAT BRAIN. H.J. Bennett*, J. Burns and K. Semba. Dept. of Anatomy & Neurobiology, Dalhousie University, Halifax, N.S., CANADA B3H 4H7.

Caffeine is a methylxanthine which has a mild stimulatory action in the CNS. Its action is thought to be mediated primarily by antagonism at adenosine receptors. Extracellular adenosine has been demonstrated to have a tonic inhibitory effect at a number of presynaptic and postsynaptic sites. Previous studies have shown that high doses (>75 mg/kg, i.p.) of caffeine increase c-fos mRNA in the striatum of the rodent brain. In order to investigate the effects of caffeine at lower doses and in extrastriatal structures, male Wistar rats were injected with caffeine (1, 5, 10 or 75 mg/kg, i.p.) or saline, perfused 2 hrs later, and their brains were processed for Fos immunohistochemistry. Basal expression of Fos was observed in a number of brain regions after saline injection. The distribution seen with 1 or 5 mg/kg caffeine was not significantly different from the basal expression. In contrast, following 10 mg/kg injection greater numbers of Fos-positive neurons were seen in the medial prefrontal, cingulate and pyriform cortices, olfactory tubercle, hypothalamic nuclei, paraventricular thalamic nucleus, supragenual nucleus, and cochlear nuclei. With 75 mg/kg caffeine, in addition to the above regions Fos-immunoreactive neurons were observed in the lateral striatum, globus pallidus, substantia nigra pars reticulata, central nucleus of the amygdala, paraventricular and supraoptic hypothalamic nuclei, lateral parabrachial nucleus, locus coeruleus, ventrolateral medullary reticular formation, and nucleus of the solitary tract. Behaviourally, increased activity was seen after 5 or 10 mg/kg, whereas activity was reduced after 75 mg/kg; no change was observed with 1 mg/kg. These results indicate that caffeine induces c-fos expression in a dose-related manner in more widely distributed brain regions than previously reported, including those involved in limbic, autonomic, and motor functions. Supported by the MRC of Canada.

810.5

ENZYMATIC HYDROLYSIS OF AGMATINE TO PUTRESCINE IN RAT BRAIN. M. Sastre*, S. Regunathan and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021.

We have recently discovered that agmatine (decarboxylated arginine), an endogenous ligand for imidazoline receptors, and its biosynthetic enzyme, arginine decarboxylase (ADC), are expressed in mammalian brain (Li et al., *Science* 263: 966-969, 1994). In bacteria, the major metabolic pathway for agmatine is hydrolysis by agmatinase to putrescine, the precursor of polyamines, and urea. We sought to determine whether agmatine can be metabolized by a similar pathway in rat brain. Agmatinase was assayed by measuring hydrolysis of guanido^{14}C-agmatine to ^{14}C-urea and putrescine and subsequent trapping of $^{14}CO_2$ released by urease from ^{14}C-urea. Incubation of guanido^{14}C-agmatine with rat brain homogenates resulted in a substantial hydrolysis of agmatine (7.6 to 11.8 nmol/hr/mg protein). Activity is reduced (up to 75%) by boiling while ~25% of total activity remained in non-homogenate controls due to non-enzymatic degradation of agmatine. With subcellular fractionation of rat brain, agmatinase activity was maximal (48.1 nmol/hr/mg protein) in the soluble fraction of the P2 pellet (synaptosomal/mitochondrial). Further fractionation of the P2 pellet resulted in enrichment of agmatinase in the mitochondrial (327.8 nmol/hr/mg protein) vs. synaptosomal (31.2 nmol/hr/mg protein) fractions. Agmatinase activity in the P2 pellets varied regionally in brain: hypothalamus (133 nmol/hr/mg protein) > hippocampus (88) > medulla (64.5) > cerebellum (47.5) > striatum (35.8) > cerebral cortex (30.2). We conclude: (a) rat brain expresses agmatinase which can convert agmatine to putrescine and urea; (b) the enzyme is soluble and associated with mitochondria; (c) the agmatine-putrescine pathway suggests a novel metabolic pathway for polyamine biosynthesis in brain.

810.6

"CAGED" CARBON MONOXIDE: MOLECULES FOR PHOTO-RELEASING FREE CARBON MONOXIDE IN SITU. Joseph P. Y. Kao, D. Weinreich†* & Paul F. Keltz. Medical Biotechnology Center, and Depts. of Physiology, and †Pharmacology & Experimental Therapeutics, School of Medicine, University of Maryland, Baltimore, MD 21201.

We report the design, synthesis, and application of NF-CO, NV-CO, and NP-CO, a family of three "caged CO" compounds —photosensitive reagents that release the messenger molecule carbon monoxide when illuminated with long-wavelength ultraviolet light. The caged CO's are useful as Na^+ or K^+ salts, which can be bath-applied or introduced into cells via patch pipets, respectively. The caged CO's can also be readily loaded into living cells by incubation with the acetoxymethyl (AM) ester forms of the reagents. The light-induced "uncaging" reaction is characterized by $t_{½} \approx 80$ μs. Carbon monoxide photoreleased from intracellular caged CO is as effective as exogenous gaseous CO in activating intracellular guanylyl cyclase.

The photosensitive "cages" in these caged CO compounds are based on *o*-nitrobenzyl chemistry—the most widely-used caging chemistry in biology. Common mercury or xenon light sources are thus sufficient to activate photorelease of CO. Because photorelease offers the dual advantages of spatial and temporal control of messenger molecule delivery, these caged CO's are ideal for applications in signalling studies.

(Supported by GM46956 and an SRIS Grant from the Univ. of Maryland School of Medicine)

810.7

A LYSINE RESIDUE OF THE CANNABINOID RECEPTOR IS CRITICAL FOR RECEPTOR RECOGNITION BY SEVERAL AGONISTS BUT NOT WIN55212-2. Z. H. Song* and T. I. Bonner. Lab. of Cell Biology, NIMH, NIH, Bethesda, MD 20892.

Lysine192 in the third transmembrane domain of the human CB1 cannabinoid receptor was converted to an alanine to study its role in receptor recognition and activation by agonists. HU-210, CP-55940, WIN55212-2, and anandamide, four cannabinoid agonists with distinct chemical structures, were used to characterize the wild-type and the mutant receptors. In 293 cells stably expressing the wild-type receptor, specific binding to [^{3}H]WIN55212-2 and inhibition of cAMP accumulation by cannabinoid agonists were demonstrated, with different ligands exhibiting the expected rank orders of potency and stereoselectivity in competition binding and functional assays. In cells expressing the mutant receptor, the binding affinity of the receptor for [^{3}H]WIN55212-2 was only slightly affected (the K_d for the mutant receptor was twice that of the wild-type), and the ability of WIN55212-2 to inhibit cAMP accumulation was unchanged. However, HU-210, CP-55940 and anandamide were unable to compete for [^{3}H]WIN55212-2 binding to the mutant receptor. In addition, the potencies of HU-210, CP-55940 and anandamide in inhibiting cAMP accumulation were reduced by more than 100-fold. These results demonstrate that lysine192 is critical for receptor binding by HU-210, CP-55940 and anandamide. Since lysine192 is not important for receptor binding and activation by WIN55212-2, WIN55212-2 must interact with the cannabinoid receptor through at least one point of interaction that is distinct from those of the three other agonists.

810.8

WHOLE CELL PATCH-CLAMP MEASURED ANGIOTENSIN II-INDUCED CURRENT CHANGES IN BRAIN SLICES F.P. Martial*, A. Goucham, S.N. Thornton, S. Nicolaïdis. Neurobiologie des Régulations, Collège de France, 11 place Marcelin Berthelot, 75231 Paris CEDEX 05, France.

Previous experiments on anesthetized rats have shown that many neurons in the *septo-preoptic continuum* are sensitive to Angiotensin (AII) and/or Losartan (Los).

Neuronal post-synaptic currents evoked by short time step depolarizations were recorded in slice preparations of the *septo-preoptic continuum* from juvenile rat brains using the whole cell patch-clamp technique. The effect of the specific non-peptidic antagonist of the angiotensin Type-1 receptor, Losartan (DUP 753), was also tested.

Rats weighed between 60 and 100 g on the day of the sacrifice. The temperature of the perifusion *milieu* was 25 ± 1°C. For each cell several step depolarization series (increasing by 20 mV) were applied under control conditions and then after bath application of AII or Los.

Preliminary results showed that at least two types of units were recorded. In the first type, the presence of AII in the bath produced an outward current composed of two distinct phases: one rapidly inactivable phase, and a second non-inactivable but saturable phase. In this type of unit Los could partially antagonize the effect of AII, and this lasted several minutes or more.

The second type of unit observed showed, under control conditions, weak, voltage dependant, rapidly inactivable, inward currents. These currents disappeared in the presence of AII but remained unaffected application of Los in the bath.

These results are consistent with several observations made previously in our laboratory with extra-cellular recordings and micro-iontophoresis as well as in behavioral studies. The variability of the responses to iontophoretic application of AII as well as the long term action of Los already observed in our laboratory were quite puzzling. Now, these whole-cell recordings show that AII should must not be seen as a mere neurotransmitter but rather as a subtle neuromodulator capable of acting on different receptors, pre as well as post-synaptically. Los, on the other hand, has a much longer, and apparently more complicated, action on these receptors.

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810.9

CHARACTERIZATION OF A LOW AFFINITY BINDING SITE ON THP-1 MONOCYTES USING PEPTIDE AND NON-PEPTIDE NEUROKININ LIGANDS. S.L. Yates*, J.M. Kocsis, and K.R. Brunden. Gliatech, Inc., 23420 Commerce Park Rd., Cleveland, OH 44122.

Substance P (SP) typically initiates signal transduction through its binding to neurokinin 1 (NK1) receptors. SP is known to increase the production of interleukin-1 from human monocytes; however, these cells are devoid of high affinity NK1 receptors. Jeurissen *et al.* (J Immunol, 1994, 152:2987) have recently identified a low affinity non-NK1 receptor on human monocytes. To further characterize the properties of this receptor, we have examined the binding of the non-peptide NK1 antagonist, L-703,606, to human myeloid leukemia (THP-1) cells. The THP-1 cells were stimulated with lipopolysaccharide for 48 hr, subsequently harvested, and membrane homogenates were prepared. Binding of ^{125}[I]L-703,606 was performed at 4°C in Tris buffer, pH 7.4, containing a cocktail of protease inhibitors. Association analysis revealed that ^{125}[I]L-703,606 binding saturated within 10 to 15 min. Unlabeled L-703,606 inhibited the binding of ^{125}[I]L-703,606 with an apparent affinity of ~200 nM. Interestingly, SP did not inhibit ^{125}[I]L-703,606 binding, nor did a variety of NK peptide antagonists and agonists. To clarify these observations, additional studies were conducted as above using ^{125}[I]SP. Unlabeled SP caused a dose-dependent inhibition of ^{125}[I]SP binding, revealing a low affinity binding site. L-703,606 and the other NK compounds tested above were able to compete for ^{125}[I]SP binding. These data provide evidence of a low affinity NK-like receptor on THP-1 cells which may have a distinct non-peptide regulatory/modulatory binding site in addition to a peptide binding site.

810.10

CLONING AND CHARACTERIZATION OF BRAIN CYSTEINE SULFINIC ACID DECARBOXYLASE. X.W. Tang, Y. Sun, C.C. Hsu, C.-Y. Yang[1] and J.-Y. Wu*. Dept. of Physiol. & Cell Biol., Univ. of Kansas, Lawrence, KS 66045, and [1]Baylor College of Med., Houston, TX 77030

Taurine is one of the most abundant free amino acids found in mammalian CNS, and is believed to play many important physiological functions. The rate limiting enzyme in taurine biosynthesis in the brain is believed to be cysteine sulfinic acid decarboxylase (CSAD). Despite its importance, little is known regarding the molecular nature and mode of regulation of CSAD. Here we report the purification of CSAD to homogeneity from porcine brain by a combination of column chromatographies of hydroxylapatite, DE-52 and Sephadex G-100 and polyacrylamide gel electrophoresis(PAGE). The purified CSAD has a molecular weight of 86-90 kDa and 43 kDa on non-denaturing gradient PAGE and SDS-PAGE, respectively, suggesting that the CSAD is a homodimer of 43 KDa. A cDNA encoding CSAD has been cloned from porcine brain cDNA library using anti-CSAD as probe. The clone was further verified by showing CSAD activity when it was expressed in bacteria. Partial DNA sequence of the CSAD cDNA has been obtained. In regard to the mode of regulation, CSAD activity was found to increase to an extent of 50%, 120%, 690% when crude synaptosomes were treated with 5 mM ATP, 0.2 mM vanadate, or 2mM EDTA/EGTA respectively. Furthermore, when crude CSAD preparations were incubated with alkaline phosphatase, CSAD activity decreased to 75% of its original value, indicating that the CSAD activity might be regulated by phosphorylation. (Supported by grants from Office of Naval Research; NIH, NS20978; and NSF, BNS-8820581)

810.11

ZINC PROTOPORPHYRIN IX (ZnPP) INHIBITS RELAXATION AND SECOND MESSENGER ACTIVATION IN THE RAT AORTA IN A MANNER DISTINCT FROM INHIBITION OF HEME OXYGENASE (HO). *Lars Ny,* Lars Grundemar and Karl-Erik Andersson, Department of Clinical Pharmacology, Lund University Hospital, S-221 85 Lund, Sweden.* Carbon monoxide (CO), which can be produced by HO through degradation of heme has been claimed to be a neuronal messenger and a regulator of the vascular tone. We examined whether the HO inhibitor ZnPP and other porphyrins affects vasodilation evoked by various agents in the rat aorta. ZnPP (0.1 mM) virtually abolished the relaxation evoked by vasoactive intestinal peptide (VIP), atrial natriuetic peptide (ANP) and attenuated the relaxation induced by acetylcholine (ACh). In contrast, ZnPP did not affect the relaxation evoked by forskolin and 3-morpholino-sydnonimin, agents which directly activate adenylate cyclase and guanylate cyclase, respectively. Also SnPP and PP attenuated the VIP-evoked relaxation. ZnPP also abolished the elevation of cAMP and cGMP levels evoked by VIP and ANP, respectively. Neither ZnPP nor PP affected the contraction evoked by phenylephrine. Thus, ZnPP inhibits relaxation induced by VIP, ANP and ACh, probably by interfering with membrane receptor-coupled dilatory signal transduction pathways. This effect does not seem to be dependent upon inhibition of HO. It appears that the lack of specificity of the HO inhibitors makes them less suitable as tools in the investigation of a messenger role of CO.

810.12

HEME OXYGENASE ISOENZYMES AND EFFECTS OF CARBON MONOXIDE IN THE FELINE LOWER ESOPHAGEAL SPHINCTER.

Lars Grundemar[1], Lars Ny[1], Bengt Larsson[1], Per Alm[2], Peter Ekström[3] and Karl-Erik Andersson[1], Departments of [1]Clinical Pharmacology, [2]Pathology and [3]Zoology, Lund University, Lund, Sweden.*

Carbon monoxide (CO) has, like nitric oxide, recently been suggested to be a gaseous messenger in the brain and periphery. The distribution of the CO producing enzymes heme oxygenase (HO)-1 and -2 were examined in the feline lower esophageal sphincter (LES). Also HO activity in homogenates and motor effects of CO in the LES were investigated. HO-2 immunoreactivity (IR) was observed in nerve cell bodies in the submucosal and myenteric plexus, nerve fibers, non-neuronal cells surrounding smooth muscle bundles, and in the endothelium of some arteries. HO-1-IR was confined to non-neuronal cells in the smooth muscle layer. HO activity, measured as CO production was demonstrated in LES homogenates. This activity was inhibited by the HO inhibitor zinc protoporphyrin. Exogenously administered CO evoked a concentration-dependent relaxation and increased cyclic GMP levels in LES strips. These results show that HO-2 as well as HO-1 are present in the LES and suggest that CO can be generated by neuronal and non-neuronal cells and may possibly have a role as a peripheral messenger.

810.13

EXPRESSION OF HO2 IN DIFFERENTIATED PC12 CELLS. E.E. Thompson* and S.H. Snyder The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Heme oxygenase (HO) catalyses the oxidation of heme to biliverdin and carbon monoxide (CO). Since CO can activate soluble guanylate cyclase, it is likely that HO plays a role in cGMP mediated signal transduction. When differentiated with NGF, PC12 cells express the neuronal isoform of heme oxygenase, HO2. HO2 enzyme activity in cellular sonicates after 6 days of NGF treatment is 5 nmol/mg/hour, which is higher than that in total brain homogenates. Also, as early as day three of NGF treatment, there is a cGMP signal by immunoassay that is sensitive to ZnPPIX, an inhibitor of HO, but not to nitroarginine. These data suggest that as PC12 cells differentiate into their neuronal phenotype, they synthesize HO2 which is used in cGMP mediated signal transduction. This makes PC12 cells an interesting model system in which to study the role of neuronal HO2.

810.14

IMMUNOCYTOCHEMICAL LOCALIZATION OF HEME OXYGENASE-2 IN RAT BRAIN. M. Yamanaka[1], Y. Nishimura*[2], R. Semba[1]. [1]Dept. of Anatomy II, [2]Dept. of Physiology II, Mie University School of Medicine, Tsu, Mie 514, Japan.

Carbon monoxide is a membrane-permeable gas that has been suggested to play a signaling role in the brain. It is formed by the enzyme heme oxygenase-2 during conversion of heme to biliverdin. In the present study, we raised antisera against the synthesized N-terminal fragment of this enzyme and studied cellular distribution of the enzyme in the rat brain by an immunocytochemical method. Heme oxygenase-2 immunoreactivity was observed only in neurons. Though immunoreactive neurons were ubiquitous to the brain, intensely stained neurons were rich in the olfactory bulb, layers III and V of the cerebral cortex, hippocampus, locus coeruleus, pontine nucleus and cerebellum. In the pyramidal cells of the hippocampus and Purkinje and basket cells of the cerebellum, immunoreactivity was manifested in the dendrite and the perikaryon rather than in the axon terminal, suggesting the function of HO-2 as a generator of a retrograde messenger.

810.15

HEME OXYGENASE-2 IN SENSORY AND AUTONOMIC NEURONS OF THE GUINEA-PIG. W. Kummer*, R. Vollerthun and B. Höhler. Inst. Anat. & Cell Biol., JLU, D-35385 Giessen, FRG

Heme oxygenase-2 (HO-2) is the major carbon monoxide (CO) generating enzyme constitutively expressed in the brain. In view of the potential role of CO as a neurotransmitter the distribution of HO-2 in the peripheral nervous system was investigated by immunohistochemistry, and the immunoreactive protein was characterized by western blotting using an antiserum raised against rat testicular HO-2. All neuronal cell bodies in sensory ganglia (trigeminal, petrosal, jugular, nodose, and dorsal root ganglia), regardless of their size, exhibited intense immunoreactivity to HO-2. Immunoreactivity was confined to the perikaryon and did not extend into the axon. Similarly, all neuronal cell bodies of sympathetic ganglia (superior cervical, stellate, inferior mesenteric, and lumbar chain ganglia), and submucosal and myenteric ganglia of the small intestine of the guinea-pig were HO-2 immunoreactive. Peripheral tissues and organs so far investigated by immunohistochemistry (visceral arteries, fat tissue, skin) were devoid of HO-2 immunoreactive axon terminals. Western blots revealed an HO-2 immunoreactive protein with an apparent molecular weight of 36 kDa in cerebellum, sympathetic and dorsal root ganglia. The present findings establish an HO-2 immunoreactive protein as an ubiquitous component of sensory and autonomic neurons in the guinea-pig. Its molecular weight corresponds to that of brain HO-2 which is 6 kDa smaller than that of testicular HO-2. The ubiquitous distribution excludes a specific association with a particular functionally defined subset of neurons. The prominent localization in the perikaryon suggests a role in the catabolism of the numerous heme proteins synthetized and utilized by peripheral neurons. (supported by the DFG, Ku 688/2-2)

810.16

BIOLOGICAL EFFECTS OF ENDOTHELIN-1 ON ASTROCYTES ARE MEDIATED BY ET_B RECEPTOR THROUGH SEVERAL G PROTEINS. S. Cazaubon[1], P. Lacombe[2]*, A.D. Strosberg[1], P.O. Couraud[1] [1]CNRS UPR 0415, Institut Cochin de Génétique Moléculaire, and [2]CNRS UA 641, Faculté de Médecine Villemin, Paris, France

Astrocytes have been shown to express endothelin-1 (ET-1) receptors functionally coupled, via different heterotrimeric G-proteins, to several intracellular pathways. It is reported here that both receptor subtypes, ET_A-R and ET_B-R, mRNAs were detected in primary cultures of astrocytes. To assess the relative contribution of each subtype in the astrocytic responses to ET-1, effects of BQ-123, an antagonist selective for ET_A-R, and IRL1620, an agonist selective for ET_B-R, were investigated. Binding experiments indicated that ET_B-R is the predominant subtype in these cells. Inhibition of the forskolin-stimulated cAMP production was observed under ET_B-R stimulation. *Bordetella pertussis* toxin pretreatment completely abolished this effect, indicating that this pathway is coupled to ET_B-R via Gi protein. Increase of tyrosine phosphorylation of cellular proteins, stimulation of mitogen-activated protein kinase (MAPK) and DNA synthesis were also found to be mediated by ET_B-R, but through PTX-insensitive G protein. IRL1620-induced MAPK activation involved the adapter proteins Shc and Grb2, and the serine/threonine-kinase Raf-1. This study reveals that the various effects of ET-1 in astrocytes are mediated by ET_B-R which couples to multiple signaling pathways via distinct G proteins.

UPTAKE AND TRANSPORTERS: MISCELLANEOUS

811.1

STUDY OF THE FUNCTIONAL ROLE OF N-LINKED GLYCOSYLATION SITES IN GAT-1 GABA TRANSPORT AND REQUIREMENTS FOR GLYCOSYLATION IN ADDITION TO THE CANONICAL SEQUENCE. J. A. Clark*. Laboratory of Cell Biology, NIMH, Bethesda, MD 20892-4090.

Isolation of Na^+/Cl^--dependent transporter cDNAs has made it possible to study the structure and function of these important proteins, with the goal of attaining a better understanding of how these carriers work. Canonical sites for N-linked glycosylation are found in the large extracellular loop that connects transmembrane domains three and four in nearly all members of this transporter family. Although the role of N-linked oligosaccharides may be trivial for the function of some proteins, the role is significant and varied in the function of many proteins. Studies have been undertaken to understand the role that glycosylation plays in GAT-1 GABA transporter function. Mutation of Asn residues 455, 460, and 463 to Gln residues results in no detectable GABA transport in intact transiently transfected CV-1 cells. The nature of this loss of transport activity will be described.

In vitro translation of a GAT-1 construct resulting in truncation of the protein at Gln residue 209 results in expression of a nonglycosylated protein. However, truncation of GAT-1 at Trp residue 285 results in a glycosylated product. These data suggest that something in addition to the canonical site is necessary for N-linked glycosylation of GAT-1.

811.2

COCAINE INHIBITS GABA TRANSPORT AT NEURONS IN THE DORSOLATERAL SEPTAL NUCLEUS (DLSN). S. Shoji and J.P.Gallagher.* Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555.

To investigate the action of cocaine, we applied cocaine to brain slices, in vitro, and conducted intracellular recordings from neurons in the DLSN. Brain slices were obtained from drug naive rats (NR) or chronically cocaine treated rats (CR, 15 mg/kg, IP, 2X daily for 14 Days). In NR, superfusion of cocaine produced a membrane potential hyperpolarization in only 50% of sampled neurons, while slightly prolonging the duration of evoked IPSPs ($GABA_A$ and $GABA_B$). On the other hand, 100% of DLSN neurons recorded from CR exhibited a hyperpolarization to cocaine (3 μM) with a significant prolongation in the duration of IPSPs. The cocaine induced-membrane potential hyperpolarization, which is due to an activation of both potassium and chloride conductances, was TTX-insensitive and persisted in zero calcium solution. Lowering the extracellular sodium (35 mM) or chloride (7.4 mM) blocked the cocaine induced-hyperpolarization. Higher concentrations of the potent GABA uptake blocker NO-711 (10 μM), which itself induced a membrane potential hyperpolarization and greatly prolonged the duration of IPSPs, blocked the cocaine induced-hyperpolarization.

These results suggest that cocaine inhibits GABA uptake in DLSN neurons. Moreover, in brains from CR, cocaine inhibition of GABA uptake is greatly potentiated compared to NR. This latter potentiating effect of chronic cocaine may be a cellular electrophysiological correlate of behavioral "SENSITIZATION". Supported by DA-07190.

811.3

MULTIPLE GABA PLASMA MEMBRANE TRANSPORTERS ARE EXPRESSED IN THE VERTEBRATE RETINA.
J. Johnson*, T.K. Chen, C. Evans, D. Rickman, and N. Brecha. Depts. of Neurobiol., Med. and Psych., UCLA and VAMC-WLA, LA, CA 90073; Dept. Ophth., St. Louis Univ., St. Louis, MO 63104.

The purpose of this study was to determine the cellular expression pattern of three GABA transporters (GATs) in the rat retina using affinity purified polyclonal antibodies directed to the C-terminus of GAT-1, GAT-2 and GAT-3. Antibody specificity was tested by preadsorption of the primary antibody with 10^{-5}M C-terminal peptides of known GABA and glycine transporters. Numerous GAT-1-immunoreactive (IR) amacrine cell bodies are in the proximal inner nuclear layer (INL). A few IR displaced amacrine and rare ganglion cell bodies are in the ganglion cell layer (GCL). GAT-1-IR processes are densely distributed to all inner plexiform layer (IPL) laminae. Weak GAT-1-IR is also present in Müller cell endfeet and processes in the outer retina. GAT-2-IR is localized to retinal pigment and ciliary epithelia. GAT-3-IR is expressed predominantly by Müller cells. Some GAT-3-IR cell bodies are found in the proximal INL and IR processes are densely distributed to all IPL laminae. These data indicate that whereas GAT-1-IR is prominently expressed by neurons, and weakly expressed by Müller cells, GAT-3-IR is prominent in Müller cells, and GAT-2-IR is confined to non-neuronal cells. GAT-1 and GAT-3 are therefore likely to mediate high affinity uptake of GABA in the retina and influence synaptic activity. In contrast, GAT-2 may participate in fluid balance in the retinal pigment and ciliary epithelia.

Supported by NEI EY 04067 and VA Medical Research Funds.

811.4

CHARACTERIZATION OF NEUROTRANSMITTER TRANSPORTER-HOMOLOGOUS cDNA CLONES IN *DROSOPHILA*.
W. S. Neckameyer,* K. Driscoll and A. Pruett. Dept. of Pharmacological & Physiological Science, St. Louis University School of Medicine, St. Louis MO 63104.

Several independent cDNA clones were previously isolated from a *Drosophila melanogaster* adult head cDNA library using a hybridization probe derived from a highly conserved region of the norepinephrine and γ-aminobutyric acid transporters (Neckameyer, Neurosci. Abstr. 19: 1122, 1993).

Sequence analysis of one *Drosophila* cDNA clone displays remarkable conservation with the cloned rat GABA transporter. Two other cDNA clones fall into the same major class by Southern hybridization analysis of *Drosophila* genomic DNA; however, these clones have different restriction enzyme patterns and mRNA transcription patterns, suggesting they are related but independent transporters. The second class of cDNAs shares homology by Southern analysis but not at the DNA sequence level. Further characterization of these cDNAs will be presented.

811.5

Mammalian Brain-Specific L-Proline Transporter: Preferential Localization of Transporter Protein to Glutamatergic Nerve Terminals in Rat Forebrain.
R.T. Fremeau, Jr.*, J. Chan, A. Pohorille, J.V. Nadler, T.A. Milner, and V.M. Pickel. Depts of Pharmacol. & Neurobiol., Duke Univ. Med. Ctr., Durham, NC 27710; and Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021

A high affinity mammalian brain-specific L-proline transporter (PROT) has been cloned. PROT is a member of the Na^+-(and Cl^-)-dependent plasma membrane transporter family that includes transporters for several neurotransmitters, osmolytes, and nutrients. Previous *in situ* hybridization studies localized rPROT mRNA to subpopulations of glutamatergic neurons in rat brain. However, no direct morphological evidence exists about the regional and subcellular distribution of the PROT protein. Thus we used an affinity-purified antipeptide antibody directed against the carboxy-terminus of this transporter for the light and electron microscopic immunolocalization of the PROT protein in rat forebrain. Immunoperoxidase labeling revealed abundant, punctate PROT immunoreactivity in forebrain regions that receive dense glutamatergic innervation including the olfactory bulb, piriform cortex, hippocampal formation (HF), amygdala, caudate-putamen (CPu), thalamus, and hypothalamus. Within HF, prominent immunolabeling was observed in the inner and outer thirds of the dentate molecular layer and in strata oriens and radiatum of the CA3 and CA1 regions. Electron microscopic immunogold detection of PROT in CPu revealed prominent labeling of axon terminals forming asymmetric, excitatory-type synapses with dendritic spines. Unexpectedly, the majority of gold particles were observed overlying vesicular organelles within the axonal cytoplasm. In most cases, at least one or two gold particles were in contact with the plasma membrane adjacent to the synapse. These findings raise the possibility of a novel presynaptic regulatory role for PROT in excitatory transmission. High affinity L-proline uptake may regulate the synthesis and/or release of glutamate in specific excitatory nerve terminals. Alternatively, high affinity uptake may limit postsynaptic actions of L-proline by reducing its extracellular concentration in synaptic and/or surrounding spaces. Supported by NIH grants NS32501, NS16064, MH42834, MH40342, DA04600, and NSF 9310965.

811.6

MOLECULAR CHARACTERIZATION OF A TAURINE TRANSPORTER CLONED FROM MOUSE RETINA.
V. Sarthy*[1], H. Sarkar[2], X. Qian[2] and H. Egal[1], Dept. of Ophthalmology, Northwestern University, Chicago, IL 60611; and Dept. Of Physiology, Baylor College of Medicine, Houston, TX 77030.

The high intracellular taurine levels in ocular tissues are maintained by high affinity, Na^+-dependent taurine transport systems. In the course of screening a mouse retina cDNA library for neurotransmitter transporters, we isolated a cDNA clone whose predicted protein sequence showed strong homology to known taurine transporters from rat brain (98.4%) and human placenta (94.7%). RNA transcribed *in vitro* from the cDNA clone induced Na^+ and Cl^--dependent, ^3H-taurine uptake in microinjected *Xenopus* oocytes. Whereas taurine uptake rate varied hyperbolically with external taurine or Cl^- concentration, the uptake rate showed a sigmoidal dependence on external Na^+ concentration. Hill plots suggested that the taurine transporter required 2 Na^+ and 1 Cl^- per taurine molecule. Taurine uptake was inhibited 50% or more by 100 μM β-alanine, hypotaurine, guanidinoethanesulfonic acid and guanidinopropionic acid. Homotaurine and glycine, however, did not block uptake. Furthermore, taurine uptake was inhibited by the phorbol ester, PMA but not by its inactive analog, 4-α-PDD suggesting a role for phosphorylation in taurine transport. Finally, *in situ* hybridization studies showed that the taurine transporter was highly expressed in the ciliary body and to a lesser extent in the mouse retina.
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811.7

LEUCINE INFUSED INTO THE BRAIN BY MICRODIALYSIS COMPETES FOR THE IN VIVO LARGE NEUTRAL AMINO ACID TRANSPORTER.
H. R. Zielke*, Y. Huang, C.L. Zielke, Peter Baab and J.T. Tildon. Dept. of Pediatrics, University of Maryland, Baltimore, MD 21201.

The effect of leucine on the interstitial concentration of the large neutral amino acids in rat brain was examined using a microdialysis delivery system and HPLC analysis of the eluant. Microdialysis probes were inserted stereotaxically into the hippocampus of rats. Leucine [0.5, 2, 5 and 10 mM in artificial CSF] was infused into the awake, free-moving rat and the eluate from the probe was analyzed for 20 amino acids. The infusion of leucine rapidly increased the concentration of phenylalanine, tyrosine, methionine and tryptophan to higher steady state levels. A 2-4 fold increase in concentration in these amino acids was observed at 10mM leucine with minor changes in other amino acids, except glutamine which doubled in concentration. The response to leucine was concentration dependent. Inclusion of 2 mM phenylalanine or tyrosine in the artificial CSF (instead of leucine) increased leucine and tyrosine (or phenylalanine with 2 mM tyrosine), methionine and tryptophan. The data suggest that leucine, known to accumulate in Maple Syrup Urine Disease, interferes with the uptake of the amino acid precursors for serotonin and catecholamine biosynthesis. The reduced uptake may explain some of the neurological and developmental defects observed in this disease. Finally, the increase in interstitial glutamine observed in the presence of leucine is consistent with the published data of Yudkoff et al. (J. Neurochem. 62:1192-1202, 1994) who showed that leucine is an amino donor for glutamine formation. (NIH grant 16596)

811.8

IMMUNOCYTOCHEMICAL ANALYSIS OF AMINO ACID TRANSPORT INTO NEURONS AND GLIAL CELLS IN THE RABBIT RETINA USING SPECIFIC ANTIBODIES AGAINST AMINO ACID ANALOGUES AND STEREOISOMERS.
D. V. Pow, D.K. Crook, I.R. Keast* and D.I. Vaney. Vision, Touch and Hearing Research Centre, Department of Physiology and Pharmacology, University of Queensland, Brisbane, Queensland 4072.

Neurons appear to be dependent upon extracellular supplies of substrates for the formation of amino acid transmitters such as glutamate and GABA, and for the formation of amino acid-derived compounds such as nitric oxide. To determine which cells in the retina exhibit high-affinity uptake of amino acid transmitters and their precursors, we have developed highly specific antibodies to molecules such as D-aspartate, D-glutamine and L-NAME; these molecules do not occur naturally in mammals, but are transported by glutamate, glutamine and arginine transporters, respectively. Thus transport of these molecules into cells can be distinguished immunocytochemically from pools of endogenous molecules. Retinae were isolated from rabbits that had been killed by an intravenous overdose of sodium pentobarbital (80 mg/kg). Retinae were incubated with test compounds (40 μM) dissolved in a physiological saline solution (Ames medium), then fixed and processed for immunocytochemistry, using 0.5 μm semithin sections. This paradigm results in high resolution labelling of specific classes of cells. Examination of serial semithin sections permitted the identification of endogenous molecules. For instance, D-arginine is strongly accumulated in a subset of retinal neurons, including some GABAergic amacrine cells; D-arginine also accumulates in a population of amacrine cells that contain neither GABA nor glycine. By contrast, D-glutamate is taken up mainly by glial cells, where it is converted into D-glutamine. This D-glutamine is then exported to subsets of neurons including the glutamatergic retinal ganglion cells. We believe that this immunocytochemical approach to demonstrating amino acid transport is a powerful tool both for analysing the cell specificity of uptake and for examining any activity-dependence of these transport systems.

811.9

DISTRIBUTION OF mRNA CODING FOR THE MAMMALIAN BRAIN-SPECIFIC L-PROLINE TRANSPORTER. T.S. McGraw*, R.T. Fremeau, Jr. and K.J. Anderson, Depts. Physiol. Sci. & Neuroscience, Univ. Florida, Gainesville, FL 32610; Dept. Pharmacol. & Neurobiol., Duke Univ. Med. Ctr., Durham, NC 27710.

The presence of a high affinity Na^+-dependent synaptosomal uptake system for L-proline (PROT) has generated interest in the possible role of this transporter in synaptic function. Initial reports on the cloning and distribution of PROT demonstrated that it was expressed in subpopulations of glutamatergic pathways in the CNS, further suggesting that this transporter may modulate excitatory synaptic transmission. We have utilized in situ hybridization to further characterize the anatomical distribution of PROT mRNA in the CNS. Oligonucleotide cDNA probes (45mer) were constructed that were complimentary to the published sequence of PROT. Probes were screened against GenBank to ensure specificity and labelled with [^{35}S]ATP for film and emulsion-based in situ hybridization. Rat brains were sectioned in the horizontal, coronal and saggital planes and hybridized with labelled probes. Control probes consisted of sense strands from the same sequence. PROT mRNA was found in high abundance in the olfactory bulb mitral cell layer, external plexiform layer neurons, olfactory tubericle and anterior olfactory nucleus. Within the thalmus, specific nuclei were labelled including the anterior dorsal, lateral posterior and lateral doral nuclei. In the hippocampal formation, intense labelling was seen in pyramidal neurons of CA1-4, while granule cells of the dentate gyrus were lightly labelled. Labelling was observed in cerebral cortical layers III and V of the somatosensory cortex. Intense labelling was seen in neurons of layers II-III of the entorhinal cortex. Diffuse labelling was seen in the brainstem and cerebellum. These findings are consistent with previous observations that PROT is found in some, but not all, glutamatergic pathways in the CNS. The localization of PROT to specific populations of excitatory neurons may provide another means to regulate excitatory neurotransmission. Supported by AG-08843, NS32501, and NSF 9310965.

811.10

CHARACTERIZATION OF ASPARTATE RELEASE VIA REVERSAL OF CLONED HUMAN EXCITATORY AMINO ACID TRANSPORTER SUBTYPES [1,2]V.R. Roettger*, [2]J.L. Arriza, and [1,2]S.G. Amara, [1]Howard Hughes Medical Institute and [2]Vollum Institute , Oregon Health Sciences University, Portland, OR

Excess glutamate release may contribute to ischemic brain damage; the mechanism for this release is probably reversal of one or more Na^+-dependent glutamate transporters (Roettger and Lipton, Soc. for Neurosci. Abst., 20, 1994). We used stably transfected HEK293 cells independently expressing three excitatory amino acid transporter subtypes (EAAT1-3) isolated from human motor cortex (Arriza et al., J of Neurosci., 14:5559, 1994) to deteermine which subtype(s) may be involved. In addition, different release paradigms were examined to determine which conditions can trigger excess release during ischemia.

Methods: Cells expressing EAAT1, EAAT2 , EAAT3, or vector alone (vector control) were loaded with ^{3}H-D-Aspartate (D-ASP) for 30'; EAAT transfected cells accumulated D-ASP at levels greater than 5-fold over that accumulated by vector control cells. Release was stimulated by exposure to either: (1) combined sodium azide (Az - 20mM) and iodoacetate (IAA - 0.5mM) which lowers cellular ATP; (2) gramicidin D (GD - 50ug/ml) which increases internal Na^+; or (3) nigericin (NIG - 20uM) which exchanges external H^+ for internal K^+; all these conditions occur during ischemia. Results: Az/IAA or GD exposure stimulated D-ASP release from transporter-transfected cells to approximately the same level for all subtypes (2-fold greater than release from vector control cells). However, NIG exposure stimulated D-ASP release from EAAT1 and EAAT2 transfected cells to a larger degree over vector control cells (>3-fold) than release from EAAT3 transfected cells (<2-fold greater than vector control cells). This suggests that EAAT subtypes may be differentially regulated by H^+ ions.

These results indicate that the stimulus required to release glutamate via reversal of uptake may differ between cloned transporter subtypes and suggest that all transporter subtypes may not be involved in ischemia-induced glutamate release to the same extent.

811.11

LOCALIZATION OF A NEUTRAL AND BASIC AMINO ACID TRANSPORTER IN RELATION TO NITRIC OXIDE SYNTHASE IN RAT FOREBRAIN. V.M. Pickel*, A. Pohorille, J. Chan, S.S. Tate, and M.J. Nirenberg, Depts. of Neurology and Neuroscience and Biochemistry, Cornell University Medical College, New York, NY 10021.

The neutral and basic amino acid transporter (NBAT) is one of two known members of a novel class of proteins involved in sodium-independent transmembrane transport of amino acids. NBAT is highly expressed in renal and intestinal brush border membranes, and has also been localized to enteric neurons and to selective neurons in brainstem and spinal cord in a distribution similar to that of nitric oxide (NO). In addition, L-arginine, the precursor for NO-synthesis is a potent NBAT substrate. Thus, to further establish the potential role of NBAT in regulation of substrate availability to NO-containing neurons, we examined the localization of a previously characterized antiserum against NBAT in relation to neurons immunolabeled for NO synthase (NOS) in rat forebrain. NBAT immunoreactive perikarya seen by light microscopy had many morphological similarities to the NOS-labeled cells including the presence of one or more processes in contact with blood vessels. Perikarya and processes containing NBAT were prominently distributed in the striatum, amygdala, and deep cortical laminae. In the striatum, electron microscopic dual labeling showed immunogold-labeling for NBAT frequently associated with plasma membranes of unlabeled dendrites. Although few of these dendrites or perikarya were dually labeled for NOS, many were directly apposed to NOS-immunoreactive processes. We conclude that NBAT may be involved in neuronal uptake of amino acids, which in turn regulates the availability of arginine and other substrates to NO neurons. (Supported by grants MH00078; MH40342; and HL 18974).

811.12

INSULIN REGULATES 14C-CREATINE UPTAKE IN GLIAL AND MUSCLE CELL LINES. Tea N. Kekelidze and Mario D. Saltarelli.* Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322.

Creatine and its phosphorylated derivative creatine phosphate (CP) play an important role in the maintenance of intracellular ATP levels. Creatine is phosphorylated by creatine kinase to form CP, which is believed to be used for the regeneration of ATP pools. An adequate supply of creatine is required for the maintenance of CP levels. Creatine is selectively accumulated from the extracellular space by a sodium-coupled high-affinity creatine transporter (CREAT) located in the cellular plasma membranes from brain, muscle, and other peripheral tissues. CREAT has been shown by recent cloning studies to be structurally related to the norepinephrine/GABA transporter family. Insulin has been shown to influence the metabolism of creatine and phosphocreatine, and rapidly alters creatine accumulation in muscle.

We have recently shown that CREAT velocity increases 5-10 fold when either C6 glioma or L6 myoblast cell lines are incubated in creatine-free medium, when compared with cells grown in the presence of 5 mM creatine (McClatchey et al. (1995), ISN Abstracts, J. Neurochem.). Northern analysis of RNA isolated from creatine-starved and creatine-fed cells showed no change in the relative amounts of CREAT RNAs, supporting the existence of post-translational regulatory mechanisms involved in the maintenance of intracellular creatine levels.

To further clarify the mechanisms involved in CREAT regulation, we assessed creatine uptake in C6 and L6 cells exposed to insulin (0.1 μM). After preincubation in serum-free medium, acute (5, 10, 30 min) insulin treatment resulted in a 40 ± 6% (X ± S.E.M.; N=5; $p < 0.002$) reduction of sodium-dependent 14C-creatine uptake, when compared with vehicle-treated control cells. These studies will allow further clarification of the role of receptor tyrosine kinases in the post-translational regulation of creatine transport activity. Supported by NINDS CIDA NS01651.

811.13

CHARGE MOVEMENT ASSOCIATED WITH GLYCINE UPTAKE. Stéphane Supplisson*, Claude Bergman. Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris, France.

Relationships between the non linear capacitive current and the steady-state current associated with glycine uptake were analyzed for two glycine transporters showing 96% identity in their primary sequences (rGLYT1b and hGLYT1b) and expressed in *Xenopus* oocytes. As for several previously cloned Na^+/coupled transporters, relaxation currents were recorded in response to voltage steps; in the presence of substrate they disappeared while steady-state currents developed. The relaxation currents are assumed to reflect electrical charge movement within the carrier associated with partial reactions in the transport cycle. For a given voltage step, the time integral of the single exponential current relaxation (*i.e.* the amount of charge (Q) displaced) is the same at the onset of the pulse and on membrane repolarization; it decreases with increasing glycine concentration ("charge masking"). The Q/V relationship can be fitted by a Boltzman equation with $z\delta$=0.5 and $V_{0.5}$=-20mV. Current relaxations show Na^+ and Cl^- dependence. Replacing Na^+ by Choline$^+$, TEA$^+$, or N-Methyl-Glucamine$^+$ decreases the amount of mobile charges by 80%. In the presence of Li^+ (which does not promote glycine uptake) Q is reduced by only 40%. Relaxation time constants show little voltage dependence; they are, however, significantly shorter for rGLYT1b than for hGLYT1b. At 20μM glycine, the ratio between the steady-state current and the amount of masked charges is independent of the voltage and found higher for rGLYT1b than for hGLYT1b. For the same amount of displaced charges, the rat transporter produces a 2 times larger steady-state current than the human transporter.

811.14

GENE EXPRESSION OF THE RAT VESICULAR ACETYLCHOLINE TRANSPORTER R. Cervini, S. Berrard*, L. Houhou, P.-F. Pradat and J. Mallet, Lab. Génétique Moléculaire de la Neurotransmission et des Processus Neurodégénératifs, UMR C9923 C.N.R.S., F-91198 Gif-sur-Yvette, France.

The gene encoding the vesicular acetylcholine transporter (VAChT) has recently been localized within the first intron of the gene encoding choline acetyltransferase (ChAT) in both nematode and mammals, including man. The conserved structure of the ChAT/VAChT locus is unique, since both genes lie in the same transcriptional orientation and both their products are required to express the cholinergic phenotype.

We have previously shown that rat VAChT is encoded by several mRNAs. Two of them share a common 5' exon (R) with one ChAT mRNA and are therefore generated by alternative splicing of a ChAT/VAChT primary transcript. We now demonstrate that the first intron of the rat ChAT gene contains two promoters, each used to generate one VAChT mRNA. We have also identified a fifth VAChT mRNA species containing the sequence between exon R and the VAChT translation initiation codon. The existence of these five forms of VAChT mRNA with different 5'-noncoding ends can now be correlated to the size diversity of the VAChT mRNAs that we previously observed by Northern analysis.

811.15

PROTON INTERACTIONS WITH THE ACETYLCHOLINE TRANSPORTER OF SYNAPTIC VESICLES. M. L. Nguyen, H. J. Carlisle* and S. M. Parsons, Department of Chemistry, University of California, Santa Barbara, CA 93106.

The acetylcholine transporter (AChT) of synaptic vesicles exchanges internal protons for cytoplasmic ACh. The effects of protons on both faces of the AChT were studied using purified *Torpedo* synaptic vesicles and a hyposmotic lysis-resealing technique. The rate of proton leak from acidified vesicles is slowed about two-fold by vesamicol, which is an allosteric inhibitor of the AChT, but it is not affected significantly by saturating ACh. This suggests that the AChT mediates a large fraction of the permeability of vesicular membrane to protons and that it "slips". When the internal pH is set at 5.1 and the external pH is varied, uptake of subsaturating ACh decreases toward 0 at lower external pH with an apparent pK_a of 7.6±0.2. The major kinetics effect of low external pH is an increased Michaelis constant for ACh, consistent with competition of ACh with a proton for binding to the outwardly oriented AChT. When the external pH is set at 7.8 and the internal pH is varied, uptake of subsaturating ACh decreases toward 0 at higher internal pH with an apparent dependence on the second power of the proton concentration and a pK_a of about 5.6. The major kinetics effect of higher internal pH is decreased V_{max} for ACh with a smaller effect on the Michaelis constant. There is no D_2O solvent kinetic isotope effect on the rate of ACh uptake, but there is substantial slowing of the rate of the proton leak through the AChT. A model for binding of a proton or ACh alternately to the transport site and a second proton to an internal allosteric activating site that mediates a leak through the AChT by site-to-site hopping will be presented.

811.16

VESAMICOL BINDING STUDIES OF THE CLONED RAT VESICULAR TRANSPORTER FOR ACETYLCHOLINE. A. Roghani*[1], S.M. Parsons[2], and R.H. Edwards[1]. [1]Dept. of Neurology, UCLA, Los Angeles, CA 90024 & [2]Dept. of Chemistry, UCSB, Santa Barbara, CA. A large body of evidence has implicated acetylcholine (ACh) in the pathogenesis of neurodegenerative disorders such as Alzheimer's disease(AD). ACh and other transmitters are stored into synaptic vesicles from which their release is regulated in response to neural activity. This storage requires active transport from the cytoplasm. Genetic studies in the nematode *C. elegans* recently identified a putative vesicular ACh transporter (*Unc-17*). This protein shows sequence homology to previously cloned vesicular amine transporters and its distribution suggests a role in cholinergic transmission. Using RT-PCR, we have recently isolated homologous transporters, first from *Torpedo californica*, and then from rat spinal cord (rVAChT) (PNAS *91*, 10620). The full length rVAChT was expressed transiently in COS cells. After 4 days of growth in culture, the cells were homogenized and high speed membrane samples prepared and used for [^{3}H]vesamicol binding assays. Incubation of membranes with various amount of vesamicol (0 to 75 nM) under equilibrium conditions resulted in high affinity and specific binding of vesamicol (Kd=5.5 nM and Bmax=12 pmol/mg protein) to membranes from the VAChT-transfected cells, but not to those transfected with vesicular amine transporter. In a separate experiment, keeping the vesamicol concentration at 1 nM, the vesamicol binding increased rapidly with time to reach maximal levels in 15 min. Repeating this experiment in the presence of ATP or CCCP did not alter the binding profile, suggesting that the vesamicol binding to VAChT did not depend on a H^+-electrochemical gradient. Furthermore, the amount of vesamicol bound to the membrane from transfected cells was significantly less when the binding was performed in the presence of ACh, but not in presence of choline or GABA. Moreover, incubation of the membrane with varying amounts of vesamicol and ACh at equilibrium showed that, as the vesamicol concentration was raised to higher and higher values, it overcame the binding inhibition by ACh in a competitive manner. The latter results are consistant with vesamicol binding to the site of substrate recognition on the VAChT protein.

811.17

PRODUCTION AND CHARACTERIZATION OF ANTI-FUSION PROTEIN ANTIBODIES TO THE RAT VESICULAR ACETYLCHOLINE TRANSPORTER. M.L. Gilmor*, C.J. Heilman, A. Roghani, N.R. Nash, H.D. Rees, H. Yi, S.M. Hersch, R.H. Edwards, A.I. Levey. Department of Neurology, Emory Univ. School of Medicine, Atlanta, GA 30322 and Department of Neurology, UCLA School of Medicine, LA., CA 90024

A cDNA for the putative rat vesicular acetylcholine transporter (VAT) has recently been cloned, but little is known about the encoded protein. We produced rabbit polyclonal antibodies to VAT in order to characterize VAT protein. A glutathione S-transferase/VAT C-terminus fusion protein was used as immunogen and for affinity-purification of antibodies. Western blot analysis revealed specific reactivity with bands in HeLa cells transfected with VAT cDNA and which were absent in mock transfected cells. In Western blots of rat brain homogenates, VAT-immunoreactive bands were predominant in striatum, neocortex and hippocampus, with low levels in cerebellum. Immunoreactivity was eliminated with preabsorbtion on the fusion protein. Immunohistochemistry demonstrated VAT immunoreactivity in neuronal perikarya in known cholinergic cell groups, while non-cholinergic neurons were not immunoreactive. Dense VAT terminal fields and fibers were present in cholinergic projection sites, including cortex, hippocampus, basolateral amygdaloid nucleus, and many other regions of the brain. In electron micrographs, VAT C-terminus reactivity was associated with synaptic vesicles in cholinergic terminals in rat striatum and hippocampus. These studies demonstrate VAT is a selective marker of cholinergic neurons, and specifically, cholinergic synaptic vesicles and axon terminals.

811.18

THE HIGH-AFFINITY CHOLINE TRANSPORTER IS MODULATED IN VIVO BY db-cAMP. V. Vogelsberg*, N.H. Neff and M. Hadjiconstantinou, M. Departments of Psychiatry, Pharmacology and The Neuroscience Program, The Ohio State University College of Medicine, Columbus, OH 43210.

The high-affinity choline transport (HACT) is considered by many researchers to be the rate-limiting step for the synthesis of acetylcholine (ACh). The mechanism(s) involved in regulation of the transporter are not understood. We studied the effect of cAMP-dependent pathways on HACT. db-cAMP was administered i.c.v. to mice and at various time points choline (Ch) uptake, ACh synthesis and choline acetyltransferase (ChAT) activity were measured in synaptosomes from hippocampus, striatum and frontal cortex. At 1 hour db-cAMP increases choline uptake in hippocampus and frontal cortex and slightly in striatum. Choline uptake remains elevated up to 6 hours later and then returns to baseline. The change in uptake is attributed to an apparent increase in V_{max}. ChAT activity was not altered in any of the brain regions studied, but ACh synthesis was increased in all areas after 1 hour treatment with db-cAMP. Cycloheximide did not prevent the increase in HACT activity at 1 hour, indicating a mechanism other than protein synthesis is involved. Administration of okadaic acid had no effect on basal or db-cAMP activated Ch uptake, while a protein kinase A inhibitor prevented the cAMP induced increase.

811.19

INFLUENCE OF ESTROGEN ON HIGH AFFINITY CHOLINE UPTAKE IN RAT BRAIN SECTIONS. M.L. Caspers*, B.E. Deverman and M.J. Fu. Dept. of Chemistry, Univ. of Detroit Mercy, Detroit, MI 48219.

Estrogen may exert a protective effect on several enzymes of acetylcholine metabolism. The uptake of choline is the rate-limiting step in acetylcholine biosynthesis. Ovariectomized female, Fischer-344 rats (2 mo) had either 17β-estradiol (0.5 or 5 mg) or placebo pellets implanted in the nape of their necks. Placebo and 17β-estradiol-treated animals were sacrificed after 2 or 3 weeks, 24μm frozen sections of the brains were prepared and treated with hemicholinium-3 (HC-3), a competitive inhibitor of the high-affinity choline transporter. Autoradiographic studies showed high levels of specific HC-3 binding in the caudate putamen (CP) and the nucleus accumbens. Computer-assisted densitometry indicated specific HC-3 binding was 65.5% of total HC-3 binding. An 11% ($P<0.04$) decrease in HC-3 binding in the CP occurred in rats treated with 0.5 mg 17β-estradiol pellets for 3 weeks when compared to the placebo-treated group. In rats receiving 5 mg 17β-estradiol pellets, a 16% ($P<0.04$) decrease in HC-3 binding in the CP was noted after 2 weeks and a 20% ($P<0.002$) decrease was seen after 3 weeks. (Supported by a grant from the Amer. Fed. Aging Res. and a gift from J. Rose.)

811.20

ELECTROGENIC Na^+-K^+ PUMP IN THE DISSOCIATED MAMMALIAN CNS NEURONS. Mitsutoshi Munakata*, Mika Fujimoto and Norio Akaike. Department of Physiology, Kyushu University Faculty of Medicine, Fukuoka 812-82, Japan.

Active Na^+-K^+ transport maintains intracellular ionic conditions and involves in the cell excitability with the distinct electrogenicity. We investigated the basic properties of the electrogenic Na^+-K^+ pump in neurons freshly dissociated from the rat neostriatum by the use of the nystatin-perforated patch technique. In current-clamp mode, ouabain (10 μM) depolarized neurons, suggesting that Na^+-K^+ pump functions well under the present experimental conditions. In the voltage-clump mode (V_H= -40 mM) with the pipette solution containing 40 mM Na^+, raising $[K^+]_o$ evoked an ouabain sensitive outward current in a concentration-dependent manner (EC_{50}=0.74 mM) under the suppression of K^+ and voltage dependent Ca^{2+} channels, Na^+-Ca^{2+} exchanger. Tl^+, Rb^+, NH_4^+ and Cs^+ also could evoke the outward current in the order of EC_{50}: $Tl^+ > K^+ > Rb^+ > NH_4^+ > Cs^+$. The pump activity had slight voltage dependency and decreased in hyperpolarized membrane potential. The pump activity was temperature dependent (Q_{10}=3.1). The sensitivity to ouabain was also temperature dependent, and the IC_{50} of ouabain was 7.07 μM at 20 °C and 1.3 μM at 30 °C. Interestingly, the pump activity was still observed even with the Na^+-free pipette solution. However, such activity reversibly diminished when Na^+ was completely removed from the external solution, suggesting that Na^+ ions are continuously leaking into the cell, resulting in activating the pump. A mitochndiral uncoupler, FCCP (1 μM), also eliminated the pump current. These results indicate that neuronal Na^+-K^+ pump functions in concert with ion dynamics across the cytoplasmic membrane.

811.21

SODIUM-GLUCOSE COTRANSPORTER IS PRESENT AT THE ABLUMINAL MEMBRANE OF THE BOVINE BLOOD-BRAIN BARRIER. W-J Lee, D.R. Peterson, V. Burmeister, C.E. M°Cormack*, E.J. Sukowski, and R.A. Hawkins. Physiology Department, Finch Univ. of Health Sciences/The Chicago Med. Sch., North Chicago, IL 60064.

The use of brain endothelial membrane vesicles is a unique approach in the study of glucose transport across the blood-brain barrier (BBB). Membrane vesicles were isolated from bovine brain microvessels and characterized as previously described by Sanchez del Pino *et al* (J. Biol. Chem. 267: 25951-25957, 1992). Luminal membrane vesicles showed enrichment of the enzyme gamma-glutamyl transpeptidase, while abluminal membrane vesicles exhibited System-A amino acid transport activity. The uptake of radiolabeled D-glucose was determined by rapid filtration. In the absence of an inwardly-directed sodium gradient, luminal and abluminal membrane vesicles showed similar initial rates of glucose uptake. However, in the presence of a sodium gradient, abluminal membrane vesicles exhibited the ability to transiently concentrate glucose above the equilibrium value (overshoot). The intravesicular glucose concentration fell back towards the equilibrium value upon dissipation of the sodium gradient. This phenomenon was not seen in luminal membranes in the presence of sodium. The addition of phlorizin, a specific inhibitor of the sodium-glucose cotransporter, abolished the overshoot in abluminal membrane vesicles. Kinetic analysis of glucose transport in abluminal membrane vesicles, showed the presence of 2 distinct carriers; a high-affinity, low capacity sodium-glucose cotransporter, and a passive low-affinity high capacity facilitative transporter. Luminal membranes possess only a passive facilitated carrier. These results indicate that glucose transport across the BBB is more complex than initially thought, and that the abluminal sodium-glucose transporter may be important in regulating glucose flux into and out of the extra-cellular fluid of the brain. (Supported by NIH grants NS31017 and DK42331).

811.22

Characterization of Na^+-Dependent P_i Uptake in Cultured Fetal Rat Cortical Neurons. M. Glinn*, B. Ni and S. M. Paul. Lilly Res. Labs., Lilly Corp. Ctr., Indpls., IN 46285.

We have recently demonstrated the existence of a brain-specific Na^+-dependent inorganic phosphate (P_i) cotransporter which is constitutively expressed in neurons of the rat cerebral cortex, hippocampus and cerebellum[1]. Characterization of Na^+-dependent P_i uptake in cultured fetal rat cortical neurons revealed that such transport is low up to DIV 11, but is fully developed after DIV 12 (when > 90% of saturable P_i uptake is Na^+-dependent). Na^+-dependent P_i uptake rates were initially increased and attained steady state more rapidly at 37 °C as compared with 22 °C or 4 °C, and were inhibited at low as compared with neutral or alkaline pH. Kinetic analyses revealed a K_m for P_i of 54 ± 12.6 μM and for Na^+ of 35 ± 4.2 μM. The V_{max} was 1.54 ± 0.48 nmoles/min/mg protein. The pH sensitivity and kinetic parameters observed for neuronal P_i transport were similar to those reported for P_i transport in renal-derived cells and membrane vesicles[2-6]. A reduction in extracellular Ca^{2+} markedly decreased (> 60%) Na^+-dependent P_i uptake, with a threshold for maximal P_i import of 1-2.5 mM $CaCl_2$. Cultures incubated in medium where equimolar concentrations of choline were substituted for Na^+ had lower levels of ATP and ADP and higher levels of AMP than did those incubated with Na^+. The largest fraction of $^{32}P_i$ imported with Na^+ was concentrated in the adenine nucleotides. Omission of glucose from the incubation medium for up to 18 hours did not affect Na^+-dependent $^{32}P_i$ uptake. However, depletion of ATP by incubation in choline-containing medium, or with metabolic inhibitors, dramatically decreased $^{32}P_i$ import rates. These data support the hypothesis that a major function of the Na^+-dependent P_i transporter is the import of P_i required for the production of high-energy compounds vital to neuronal metabolism.

811.23

REGIONAL DISTRIBUTION OF THE Na^+/Cl^--DEPENDENT "ORPHAN" TRANSPORTERS, RXT1 AND V-7-3-2, IN THE RAT CENTRAL NERVOUS SYSTEM. J. Masson, Z. Aïdouni, M. Pohl, B. Giros, M. Hamon and S. El Mestikawy*. INSERM U288, faculté de Médecine Pitié-Salpêtrière, 75013 PARIS, France

Rxt1 and V-7-3-2 are two members of the membrane-bound protein family which includes monoamines (dopamine, noradrenaline and serotonine)- and aminoacids (GABA, glycine, proline and taurine)-transporters. However, the substrates of Rxt1 and V-7-3-2 have not yet been identified. Northern blot studies already demonstrated that these two "orphan" transporters are exclusively synthesized in the CNS. Further studies on regional distributions of mRNA encoding Rxt1 and V-7-3-2 were presently performed by means of in situ hybridization with specific [^{35}S]cRNA antisense probes. In general, both distributions superimposed each other with high to moderate densities of Rxt1 and V-7-3-2 mRNAs in the hippocampus, cerebellum, pontine nucleus, olfactory tubercle and bulb, and spinal cord. However, differences were also noted since Rxt1 mRNA was abundant in the thalamus whereas V-7-3-2 mRNA was hardly detected in this region. Furthermore, within the substantia nigra, mRNA encoding V-7-3-2 was found exclusively in the pars reticulata whereas that encoding Rxt1 was visualized only in the pars compacta. At the hippocampal level, the pyramidal (CA1-3) and granular cell layers were found to express both mRNAs, but only Rxt1 mRNA was observed in the hilus of the dentate gyrus. Complementary immunohistochemical studies with specific polyclonal antibodies showed that the Rxt1 protein was concentrated in brain regions receiving glutamatergic and/or GABAergic inputs. At the ultrastructural level, Rxt1 was found to be associated exclusively with nerve endings. Current studies on the cellular and subcellular distributions of the V-7-3-2 protein with specific antibodies should help in further assessing the differences versus similarities between these two "orphan" members of the same transporter family.

SECOND MESSENGERS: KINASES

812.1

A NOVEL KINASE (DLK) IS EXPRESSED IN NEURONS. M. Mata*[1], G. Jiang[1], D.J. Fink[1], and L.B. Holzman[2]. [1]Department of Neurology, University of Pittsburgh, Pittsburgh, PA 15261, and [2]Department of Medicine, University of Michigan, Ann Arbor, MI 48109.

DLK (dual leucine zipper bearing kinase) is a serine-threonine protein kinase in the family of mixed lineage kinase that was identified and found in brain, kidney, and ovary (Holzman et al. 1994).

Non-radioactive in situ hybridization with a riboprobe generated from a PstI (1790-3460) cDNA fragment demonstrated a high level of expression of DLK in most neurons of the cerebral cortex, dentate gyrus and CA regions of hippocampus, basal ganglia and brain stem nuclei, cerebellar Purkinje cells, spinal cord and dorsal root ganglion neurons.

Western blot analysis of protein from cerebral cortex, hippocampus, brain stem and spinal cord, using an antibody to the carboxy terminal region of the fusion protein revealed a 130 kd protein corresponding to the translation of the DLK mRNA. Sub-cellular fractionation of tissue from rat cerebral cortex revealed that DLK appeared to be associated with the 100,000g membrane fraction, despite lacking a transmembrane domain, and is found enriched in synaptosomes isolated from rat forebrain.

In brain aggregating cultures, DLK appeared as a doublet by reducing SDS-PAGE electrophoresis. Inhibition of serine-threonine protein phosphatases by okadaic acid (5nM and 200nM) reduced its electrophoretic mobility suggesting that DLK phosphorylation is probably under the regulatory control of PP1.

This results suggest that DLK is synthetized in neurons, is membrane associated, and found in synaptosomes. DLK may play a role in signal transduction a the synapse.

812.2

Role of C1 and C2 domains in the *cis*-fatty acid activation of protein kinase C. Feleke Eshete, Hyun Chung and Kentaro Murakami*. Department of Biochemical Pharmacology, SUNY-Buffalo, NY 14260

Protein kinase C (PKC) has been shown to participate in the regulation of synaptic plasticity in the brain. *cis*-Unsaturated fatty acids (cFA) such as arachidonic acid activate PKC independently or synergistically with diacylglycerol (DAG) *in vitro*. Recent studies using slice preparations showed that the synergistic activation of PKC by cFA and DAG is also operative in the hippocampus (Chen and Murakami, Neuroscience, in press). A tandem repeat of cysteine rich zinc finger-like sequence present in the C1 regulatory domain in PKC has been considered to be the DAG interaction site, as atypical PKC such as PKC ζ which lacks one of these cysteine rich domains is insensitive to phorbol esters and DAG. The nature of interaction of cFA with PKC and the basis for the synergistic interaction with DAG in the activation of PKC is not yet known. To address these questions, we have generated PKC mutants in which C1 or C2 regulatory domain is deleted and expressed in COS-7 cells. Comparison of the biochemical characteristics of these mutated enzymes with wild type PKC shows that the alteration of the regulatory domain significantly affects the sensitivity of PKC to cFA. Further studies are underway to determine the role of C1 and C2 domains in synergy of cFA with DAG, translocation to the membrane and substrate specificity. (Supported by NIH MH48973)

812.3

PURIFICATION AND CHARACTERIZATION OF PKMζ, THE CATALYTIC FRAGMENT OF THE PROTEIN KINASE C ζ ISOFORM. E. Sublette*, S. Hrabetova, T.C. Sacktor. Depts. of Pharmacology and Neurology, State University of New York Health Science Center, Brooklyn NY 11203.

We have previously described a brain-specific, constitutively active carboxy-terminal fragment of PKCζ (PKM), which has been implicated as an important element in hippocampal long-term plasticity: levels of PKMζ increase in LTP (Sacktor *et al.* PNAS **90**: 8342, 1993) and decrease in LTD (see Hrabetova and Sacktor, these abstracts). Physiological experiments with chelerythrine, a specific inhibitor of the catalytic domain of PKC, provide further evidence that PKMζ is required for the maintenance of both LTP and LTD (*ibid.*).

Here we present a characterization of rat brain PKMζ, purified with DEAE-Sepharose, heparin-Sepharose and hydroxyapatite column chromatography. PKMζ was isolated from all other PKC isoforms, as well as from full-length PKCζ. PKMζ shows catalytic activity toward a number of substrates, including Aε pseudosubstrate peptide and histone IIIS. Inhibitory properties of chelerythrine are also examined with respect to PKMζ. An understanding of the characteristics of PKMζ may lead to more precise approaches for elucidating the function of this molecule in long-term synaptic plasticity.

812.4

ENDOGENOUS FACILITATION OF NORADRENALINE RELEASE FROM SYMPATHETIC NERVES THROUGH PHOSPHOLIPASE C GENERATION OF DIACYLGLYCEROL AND ACTIVATION OF PROTEIN KINASE C. H. Majewski*, A. Hoare & T.V. Murphy Prince Henry's Institute of Medical Research, P.O. Box 5152, Clayton, Victoria 3168, Australia.

We have previously shown that inhibition of protein kinase C (Musgrave & Majewski, 1989; Naunyn Schmiedeberg's Arch. Pharmacol. 339, 48-53) or protein kinase C down-regulation (Foucart et al., 1991 Molec Neuropharmacol. 1, 95-101) attenuates high but not low frequency release of noradrenaline from sympathetic nerves, suggesting that high output noradrenaline release is maintained by endogenous activation of protein kinase C. The present study was to investigate this hypothesis further in rat atria whose transmitter stores were radiolabelled with [^{3}H]noradrenaline. When the atria were field-stimulated at 10 Hz for 5, 10 or 15 s, there was a progressive increase in noradrenaline release and the protein kinase C inhibitor polymyxin B (21 μM) inhibited noradrenaline release at the long (10 and 15 s) but not the short (5s) duration stimulation. This suggests that activation of protein kinase C increases during the stimulation train. The phospholipase C inhibitor U73122 (3 μM) significantly inhibited noradrenaline release at the longer trains and polymyxin B added with U 73122 had no further effect suggesting that both drugs operate through the same pathway. The diacylglycerol kinase inhibitor R-59949 (1 μM), which prevents the breakdown of endogenous diacylglycerol, significantly elevated noradrenaline release evoked by the longer stimulations. However, in the presence of polymyxin B, R-59949 had no effect on noradrenaline release suggesting the involvement of protein kinase C. From these results we suggest that during a train of stimuli there is progressive activation of phospholipase C which results in diacylglycerol formation which in turn activates protein kinase C to facilitate noradrenaline release.

812.5

A NOVEL MEK KINASE ACTIVATES TWO DISTINCT MAP KINASE SIGNALING CASCADES. E.M. Elliott*, J.L. Blank, P. Gerwins, S. Sather, and G.L. Johnson. Div. Basic Sci., Natl. Jewish Ctr., Denver, CO 80206

MAP kinase (mitogen-activated protein kinase) activation defines a kinase cascade initiated by extracellular ligands binding to particular growth-factor and G_i-coupled receptors. A novel component of this kinase cascade termed MEKK2 (MAPKK kinase) has been cloned and sequenced. Two in-frame termination codons are located in the 5′ untranslated region followed by an initiation methionine. The cDNA encodes a protein of 619 amino acids, corresponding to a molecular size of 69 kD.

The MEKK2 protein was expressed in HEK 293 cells to determine its role in the MAP kinase signaling cascades. Expression of MEKK2 activates MEK1 and MKK4 (both MAPKK) and ERK1 and JNK (both MAPK). Thus, MEKK2 activates two distinct MAP kinase cascades: (1) MEK1→ ERK1 and (2) MKK4→ JNK. Both ERK and JNK activate transcription factors thereby completing the transduction of extracellular signals to the nucleus for the control of specific gene transcription.

812.6

ISOZYMIC DIFFERENCES IN THE INACTIVATION OF CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II DURING AUTOPHOSPHORYLATION. A. Hudmon*, S. J. Kolb, J. Aronowski† and M. N. Waxham. Dept. of Neurobiology and Anatomy, †Dept. of Neurology, University of Texas Health Science Center, Houston, Texas 77025.

Purified forebrain Ca^{2+}/calmodulin-dependent protein kinase II (CaM-kinase) undergoes a time-dependent inactivation that is sensitive to the conditions of autophosphorylation. We extend these findings by demonstrating that isozymes of CaM-kinase containing different ratios of alpha (50 kDa) and beta (60 kDa) subunits produce differential time-courses of enzyme inactivation during conditions of autophosphorylation. Purified forebrain CaM-kinase (3:1, alpha:beta) loses approximately 80% of its activity, while purified cerebellar CaM-kinase (1:4, alpha:beta) was observed to lose less than 30% of its activity during autophosphorylation in identical conditions. This observation was extended to both cytosolic preparations and crude homogenates of forebrain and cerebellar enzyme. Recombinantly expressed alpha or beta subunits of CaM-kinase undergo alterations in enzyme activity that parallel the dominant isozymic traits associated with forebrain and cerebellar CaM-kinase. These data indicate that differences in the ratio of alpha and beta subunits composing the holoenzyme produces isozymes of CaM-kinase with different apparent rates of inactivation, indicating that the beta subunit is not as susceptable to autophosphorylation-associated enzyme inactivation as the alpha subunit.

812.7

DOMAIN ANALYSIS OF ASSOCIATIONS BETWEEN CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II (CaMKII) SUBUNITS USING A YEAST TWO-HYBRID SYSTEM S.J.Kolb*, A.Morris†, A.Hudmon, and M.N.Waxham Dept. of Neurobiol. and Anatomy, †Dept. of Integrative Biology, Univ. of Texas Med. Sch. Houston, TX 77030.

CaMKII subunits form functionally important protein-protein interactions with other CaMKII subunits to form holoenzymes. We have analyzed the association of the two major neuronal isoforms, α and β in intact cells using a yeast two-hybrid system. Expression of these subunits in mammilian cell culture has suggested, in one study (Yamauchi et al., (1989) JBC, 264 p19108) that β subunits do not polymerize unless coexpressed with α. We detect α–α, α–β, and β–β associations between hybrid proteins in yeast. The detection of the β–β association is supported by electron microscopy studies involving purified subunits from rat brain that have suggested that pure α-containing holoenzymes and pure β-containing holoenzymes exist (Kanaseki et al (1991) JCB 155, p1049). By deletion analysis, we have confirmed that the C-terminal domain is essential and sufficient for subunit association. Moreover, associations between different domains within the same subunit, including the unique domain in the β subunit, are currently under investigation. Our findings suggest that the yeast two-hybrid system is a useful addition to current techniques designed to study the interactions within CaMKII subunits and holoenzymes.

812.8

NMDA RECEPTOR ACTIVATION LEADS TO PHOSPHORYLATION OF ELONGATION FACTOR-2: A POTENTIAL ROLE FOR NMDA RECEPTORS IN CONTROLLING PROTEIN SYNTHESIS. A.J. Scheetz*, A. Nairn# and M. Constantine-Paton · Yale University, Department of Biology and # Rockefeller University.

We are studying NMDA receptor-stimulated protein phosphorylation in the developing tadpole tectum, where structural synaptic plasticity is prominent. Using an in situ phosphorylation assay, we have previously detected 5 proteins whose phosphorylation is stimulated by NMDA receptor activation. One of these proteins, NARPP-90, has an isoelectric point and molecular mass similar to that of elongation factor 2 (EF-2), which is an enzyme that catalyzes the translocation of peptidyl-tRNA from the A site to the P site on the ribosome during protein synthesis. Phosphorylation of EF-2 by a novel calcium and calmodulin-dependent kinase (CaM kinase III) eliminates EF-2's ability to support translation and is associated with mitogenic transformation in some non-neuronal cells. Using antibodies specific to the phosphorylated form, we have examined whether EF-2 becomes phosphorylated when NMDA receptors are stimulated in the tadpole retinotectal projection. Stimulation of tecta with 50 μM NMDA + 10 μM glutamate (NMDA treatment) caused a minimum of 30-fold increase in phospho-EF-2 as detected by western blotting. Furthermore, like NARPP-90, EF-2 phosphorylation was not stimulated by application of either 60 μM AP-5 + 10 μM glutamate or 10 μM glutamate alone. As with NARPP-90, NMDA treatment of adult frog tecta, where synaptic plasticity is greatly attenuated, does not lead to EF-2 phosphorylation. Finally, western blotting of 2-dimensional gels with a pan-EF-2 antibody revealed that EF-2 comigrates with NARPP-90. These data indicate that NARPP-90 and EF-2 have similar properties and may be the same protein. They also suggest that NMDA receptor stimulation may transiently prevent protein synthesis which could be permissive for the transition of young synapses from an immature state to a more stable form.

812.9

CLONING AND EXPRESSION OF A NOVEL BRAIN-SPECIFIC P21-ACTIVATED PROTEIN KINASE (PAK2). K.J. Ivins*, M.R. Kozlowski, S.P. Manly, N. Freshney, L. Feig, R.L. Neve. Molecular Neurogenetics Laboratory, McLean Hospital, Belmont MA 02178; Geron Corporation, Menlo Park, CA; Tufts University, Boston, MA; Bristol Myers Squibb PRI, Wallingford, CT.

The p21 GTP-binding protein (p21) ras mediates the activation of the MAPK pathway following stimulation of extracellular growth factor receptors. The ras-related p21s of the rho family (rho, rac, and cdc42) mediate parallel pathways of signal transduction that are thought to modulate cytoskeletal function; but the components of these pathways have not been identified. We have cloned a rat hippocampal cDNA encoding a novel 61-kD p21 activated kinase (PAK2) that is 83% identical to a previously identified p65 PAK (Manser et al., Nature 367:40, 1994). RNA blot analysis showed that the 11 kb RNA encoding PAK2 is preferentially expressed in the nervous system. At least 4 alternatively spliced 5' UTR sequences have been identified, suggesting possible translational control of PAK2 expression. In situ hybridization histochemistry (ISSH) demonstrated that PAK2 RNA is widely distributed in adult rat brain, with high levels in hippocampus, amygdala, cortex, and the raphe nucleus; moderate levels in thalamus; and low levels in the caudate nucleus and cerebellum. In the E18 rat nervous system, PAK2 RNA is expressed at high levels in spinal cord and dorsal root ganglia as well as in brain, suggesting that PAK2 may also have a role in development. In pull-down assays using GST-p21 fusion proteins, ^{35}S-PAK2 was precipitated by GST-rac1-GTPγS and by GST-cdc42-GTPγS, but not by GST-rhoA-GTPγS or by any of the GST-p21 fusion proteins loaded with GDPγS. These results suggest that GTP-bound rac1 and cdc42 interact directly with PAK2. Furthermore, myc-tagged PAK2 expressed in COS-7 cells was immunoprecipitated with anti-myc antibody. Immunoprecipitated myc-PAK2 phosphorylated myelin basic protein in the presence of rac1-GTPγS and cdc42-GTPγS, but not in the presence of rac1-GDPγS or cdc42-GDPγS. Therefore, PAK2 is a functional kinase likely to be involved in neuronal signal transduction mediated by rac1 and cdc42.

812.10

ATP-EVOKED INOSITOL PHOSPHATE FORMATION THROUGH ACTIVATION OF P_{2U} AND P_{2Y} PURINERGIC RECEPTORS IN ASTROCYTES: REGULATION BY PKC SUBTYPES α, δ AND θ. C.C. Chen* and W.C. Chen. Institute of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan.

ATP-induced phosphoinositide (PI) hydrolysis were studied in cultured astrocytes. To characterize the P_2 purinergic receptor-mediated effects of ATP, subtype specific agonists 2-methylthio ATP (2-MeSATP), UTP and α,β-methylene ATP were comparatively studied. ATP, UTP or 2-MeSATP induced a dose-dependent increase of inositol phosphate (IP) accumulation, α,β-methylene ATP had no effect. Cross-desensitization experiments indicated that ATP interacts with both P_{2U} and P_{2Y} receptors. The effect of ATP, UTP or 2-MeSATP was inhibited markedly by pretreatment of cells with pertussis toxin (PTX). All these results suggested that ATP interacts with P_{2U} and P_{2Y} receptors coupled to PLC through PTX-sensitive G protein to mediate PI response in astrocytes. Short-term (10 min) treatment of cells with 1 μM TPA attenuated ATP, UTP and 2-MeSATP-induced PI breakdown, however, long-term (24 h) pretreatment resulted in marked potentiation of ATP and UTP responses and restoration of 2-MeSATP response. Further analyze the effect of TPA, 10 and 90 min pretreatment attenuated ATP and UTP-induced PI breakdown, but this inhibitory action was lost after 3 h treatment. Both 6 and 24 pretreatment resulted in a marked potentiation. Western blot analysis showed translocation of PKCα, PKCδ and PKCθ from the cytosol to the membrane following 10 and 90 min treatments and restoration to basal levels in the membrane fraction was seen after 3 h treatment. On the other hand, partial and complete down-regulation of these two isoforms were seen after 6 and 24 h treatment, respectively. However, PKCη was translocated but not down-regulated by TPA. These results suggested that PKCα, PKCδ and PKCθ but not PKCη may exert a tonic inhibition on P_{2U} receptor-mediated PI turnover in unstimulated astrocytes.

812.11

PHORBOL 12,13-DIACETATE (PDAc) CAN MIMIC THE NEURONAL EXCITATORY ACTION OF PHENYLEPHRINE (PhE). L.-M. Kow*, S.N. Carson, and D.W. Pfaff. The Rockefeller University, 1230 York Ave., New York, NY. 10021.

PhE can facilitate lordosis in female rats primed with estrogen and excite neurons in hypothalamic ventromedial nucleus (VMN) through the activation of α_1-adrenergic receptors, which are coupled to the phosphoinositol second-messenger pathway. We previously showed that PhE's behavioral effect could be mimicked by TPA (Brain Res. 660:241, 1994), and now used in vitro electrophysiological approach to see if the same also applied to its neuronal action. When the activity of single units recorded from VMN in hypothalamic slices was excited by PhE, a phorbol ester or related agent was infused into the chamber bathing the slice. Applications of PDAc excited 8 of the 10 (8/10) PhE-excited neurons at 10 μM, 3/5 at 5 μM, 2/3 at 2.5 μM and 5/8 at 1.0 μM. However, as the concentration decreased, the excitation became less steep and the latency to excitation prolonged. In contrast, applications of inactive phorbol esters (0/12 units at 10 μM, 0/2 at 5 μM and 0/3 at 1 μM) or the solvent DMSO (0/7 at 0.043% for 10 μM phorbol esters) was never effective. Surprisingly, administration of 12-O-tetradecanoyl phorbol 13-acetate (TPA) excited only 1/17 units at 10 μM and 0/5 at 5 μM. To see if the activation of protein kinase C (PKC) was involved, hypothalamic slices were incubated with phorbol esters or control agents at 34-37°C for ≥18 hours. In slices incubated with PDAc (10 or 20 μM) to deplete PKC, PhE excited 4/13 neurons. This responsiveness is significantly lower than the 9/12 and 6/8 from slices incubated with the inactive 4α-PDAc (10 or 20 μM) or DMSO (0.043%), respectively. Thus, the excitatory action of PhE can be mimicked by PDAc but not by TPA, and the action appears in part to involve the activation of PKC. PDAc and TPA might differ in their effectiveness and/or selectivity of PKC isoform activation. (Supported by PHS NS30824 from NINDS)

812.12

MUSCARINIC RECEPTOR ACTIVATION POTENTIATES LITHIUM-INDUCED DOWN-REGULATION OF THE PKC SUBSTRATE MARCKS IN IMMORTALIZED HIPPOCAMPAL CELLS. R.H. Lenox[1,2,3]* and D.G. Watson[1]. Departments of Psychiatry[1], Pharmacology[2] and Neuroscience[3], University of Florida College of Medicine, Gainesville, FL 32610.

We previously reported that chronic exposure of an immortalized hippocampal cell line (HN33 cells) to lithium chloride (1-10mM) produces a dose-dependent down-regulation of the PKC substrate MARCKS (**M**yristoylated **A**lanine-**R**ich **C**-**K**inase **S**ubstrate). Additionally, it was determined that the lithium-induced reduction in MARCKS protein was dependent upon the concentration of inositol, and was reversed in the presence of high inositol concentrations. In the present study we have examined the effect of muscarinic receptor activation on the expression of MARCKS protein in lithium-treated HN33 cells. Using [^{3}H]NMS binding we have determined that HN33 cells express muscarinic receptors (B_{max}: 48 fmol/mg protein) which are coupled to an IP_3/DAG response. Carbachol stimulation of [^{3}H]inositol-loaded HN33 cells produces a dose-dependent accumulation of inositol phosphate in the presence of lithium chloride. When HN33 cells were exposed to lithium chloride (1-5mM) under limiting inositol conditions, addition of 1mM carbachol significantly potentiated the lithium-induced down-regulation of the MARCKS protein. The magnitude of the MARCKS protein down-regulation following carbachol stimulation was greater in the membrane-bound fraction relative to the soluble fraction. Lithium's proposed action on PKC-mediated events, such as expression of MARCKS protein in brain, appears to be related to inositol concentration and receptor mediated signalling through the IP_3/DAG pathway. (Supported by NIMH grant MH50105).

812.13

CYCLIC GMP-DEPENDENT PROTEIN KINASE TYPE II EXPRESSION IN THE RAT BRAIN; AN RT-PCR AND *IN SITU* HYBRIDIZATION STUDY. A. El-Husseini*, C. Bladen and S. R. Vincent. Division of Neurological Sciences, Department of Psychiatry, The University of British Columbia, Vancouver, Canada, V6T 1Z3.

Cyclic GMP is produced in neurons through the activation of cell surface guanylyl cyclases by the atriopeptides, and soluble guanylyl cyclase by nitric oxide. Cyclic GMP can alter cellular responses by regulating many proteins including cGMP-dependent protein kinases, phosphodiesterases and ion channels. Immunohistochemical studies have shown that cGMP-dependent protein kinase type I is expressed in Purkinje cells and in the basal ganglia. We have used the reverse-transcription-polymerase chain reaction (RT-PCR) and *in situ* hybridization techniques to examine the expression of cGMP-dependent protein kinase type II (cGK II) in the brain. A PCR product with the size predicted from the cDNA for cGK II was detected in various regions of the brain, with highest expression in the thalamus. The amplified product of this cDNA was subcloned, sequenced and shown to be cGK II. *In situ* hybridization with riboprobes derived from the cloned cGK II PCR product indicated that this kinase was highly expressed in the outer layers of the cortex, the septum, amygdala and olfactory bulb with highest levels in the thalamus. High amounts of cGK II mRNA were also found in specific brain stem loci including the medial habenula, the subthalamic nucleus, the locus ceruleus, the pontine nucleus, the inferior olivary nuclei, and the nucleus of the solitary tract. Only low levels of cGK II mRNA were detected in the striatum, cerebellum and hippocampus. These data suggest that the effects of guanylyl cyclase activators, such as nitric oxide and the atriopeptides, in various regions of the central nervous system may be mediated through cGK II, which is widely expressed in the brain.

812.14

NITRIC OXIDE REGULATES CYCLIC GMP-DEPENDENT PROTEIN PHOSPHORYLATION IN RAT BRAIN. C. Bladen, A. El-Husseini and S.R. Vincent.* Division of Neurological Sciences, Department of Psychiatry, The University of British Columbia, Vancouver, B.C. Canada V6T 1Z3.

Nitric oxide acts via its receptor, soluble guanylyl cyclase, to increase cGMP levels and regulate various proteins including cGMP-dependent phosphodiesterases, ion channels and protein kinases. Two cGMP-dependent protein kinases have been identified in the mammalian brain, Type I, in Purkinje cells and the basal ganglia, and the recently identified Type II, which is more widely expressed throughout the brain, particularly in the thalamus (El-Husseini et al., *J. Neurochem.* in press). We have compared the regulation of protein phosphorylation in the cerebellum and thalamus by the NO-cGMP signal transduction system. Phosphorylation experiments were carried out *in vitro* on the soluble and particulate fractions of both these tissues using γ-^{32}P-ATP, and labeled proteins were examined following separation on 5-20% SDS-PAGE and exposure to X-ray film. One group of animals was injected with 20 mg/kg 7-Nitroindazole, 1 h prior to preparation of the fractions for protein kinase assays, in order to inhibit endogenous NO production in the brain. This treatment dramatically decreased the *in vitro* phosphorylation of a soluble 72kD protein. This appears to represent the autophosphorylation of the Type II cGMP-dependent protein kinase, since it was unaffected by *Rp*-8-bromo-cGMP, which is an inhibitor of the Type I, but a weak agonist of the Type II kinase. In the thalamus, this autophosphorylation could be restored, and indeed, significantly enhanced, by the addition of cGMP to the phosphorylation reaction. The labeling of this protein was even greater in the presence of 8-bromo-cGMP or cGMP plus IBMX. Labeling of a particulate protein of 46 kD was also reduced in both tissues following inhibition of NO synthase, and this could again be reversed by exogenous cGMP or 8-bromo-cGMP. These results indicate a role for endogenous NO in the regulation of protein phosphorylation by Type II cGMP-dependent protein kinase in the brain.

812.15

STAUROSPORINE, Gö-6976, AND K-252a ENHANCED KCl-EVOKED RELEASE OF 3H-NE FROM HUMAN NEUROBLASTOMA SH-SY5Y CELLS. D.B. Hawver*, A. Rashid, G. Chen, H.K. Manji, and W.Z. Potter, Section of Clinical Pharmacology , Experimental Therapeutics Branch, NIMH, and PRAT Program, NIGMS, NIH, Bethesda, MD 20892.

To explore the role of kinases in the regulation of neurotransmitter release, we have studied the effects of various kinase activators and inhibitors on the release of 3H-NE from cultures of human neuroblastoma SH-SY5Y cells. We found that the non-specific kinase inhibitor, staurosporine (STR), strongly enhanced the amount of 3H-NE released during a 7 min incubation with 100 mM KCl, without affecting basal release. Maximal effect required a preincubation of 1 hour and a dose of 50-70 nM. Two STR analogues, Gö-6976, and K-252a, similarly enhanced release. Another analogue, GF-109203X, thought to be a more specific inhibitor of Protein Kinase C (PKC), inhibited 3H-NE release at 2 μM. The PKC activators, PMA and thymeleatoxin (THM), enhanced KCl-evoked release when preincubated 14 min at 100 nM and 1 μM, respectively, and these effects appeared to be additive to those of STR, Gö-6976, and K-252a, suggesting that the activators and inhibitors may act via separate pathways. Moreover, downregulation of PKC isozymes α and ε by 24 hr treatment with 100 nM PMA or 1 μM THM abolished the enhancement of release by PMA or THM, but left intact the effects of STR, Gö-6976, and K-252a. Twenty-four hr treatment with 1 μM PMA, however, did abolish the enhancement induced by these agents, implying that there may be a PKC-sensitive step in their mechanisms, also. Finally, since both KN-62 (10 μM), a CaM Kinase II inhibitor, and Genistein (50 μM), a tyrosine kinase inhibitor, significantly inhibited KCl-evoked 3H-NE release, it appears that multiple kinases are involved in the regulation of transmitter release in this system.

SECOND MESSENGERS V

813.1

ACTIVATION OF THE PERIPHERAL CANNABINOID RECEPTOR (CB2) INHIBITS ADENYLATE CYCLASE

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Two types of cannabinoid receptors have been recently cloned. The brain cannabinoid receptor (CB1) and the peripheral cannabinoid receptor (CB2). The CB2 receptor was shown to be expressed in the HL-60 promyelocytic leukemia cell line after differentiation with TPA and it is not present in brain (Munro et al., Nature 365:61-65). Activation of CB1 with tricyclic cannabinoids or with endogenous cannabinoid ligands, the anandamides, was shown to inhibit adenylate cyclase (AC) activity in rat brain homogenates, in $N18TG_2$ neuroblastoma cells, and in cells transfected with CB1. We have recently established a stable CHO cell line that expresses CB2. Using this cell line we found that the receptor binds a variety of tricyclic cannabinoid ligands as well as anandamides. In addition, we found that activation of CB2 leads to inhibition of AC (stimulated by forskolin, PGE_1, or cholera toxin). The maximal level of inhibition was approximately 50%. The EC_{50} values were 3, 47, and 350 nM for HU210, HU293a, and HU293 respectively. The inhibition of AC by cannabinoids was abolished by pretreating the cells with 100 ng/ml pertussis toxin indicating that CB2 couples to the G_i/G_o family of GTP binding proteins. *Supported by a grant from the Forschheimer Center for Molecular Biology.*

813.2

TRANSMEMBRANE SIGNAL TRANSDUCTION SYSTEMS MEDIATING CHOLINERGIC REM SLEEP GENERATION. ML Capece, MA Fleegal, HA Baghdoyan, and R Lydic*. Department of Anesthesia, Pennsylvania State University, College of Medicine, Hershey, PA 17033

The cholinergic regulation of REM sleep has been studied in detail, yet the complex signal transduction pathway(s) responsible for REM sleep generation are unknown. Considerable data show that muscarinic receptors in the medial pontine reticular formation (mPRF) are important for REM sleep generation (*NeuroReport* 5:1631,1994). Most recently, Gi and Gs-like G-proteins have been shown to act as transducers in the ability of mPRF carbachol administration to cause a REM sleep-like state (*Am. J. Physiol.* 269:(in press),1995). As an initial step toward identifying the second messengers involved in REM sleep generation, the present study is testing the hypothesis that increasing cAMP in the mPRF will block cholinergic REM sleep generation. Cats (N=3) were implanted with standard electrodes for measuring states of waking, non-REM and REM sleep and with guide tubes for mPRF drug administration. Polygraphic recordings were made for 2 hrs following mPRF microinjection (n) of saline (control, n=13); carbachol (8.7mM, n=12); forskolin, an activator of adenylate cyclase and a stimulator of cAMP (7mM, n=8); carbachol following forskolin pretreatment (n=9); dibutyryl cAMP, a cAMP analog (87mM, n=6); and carbachol following dibutyryl cAMP pretreatment (n=6). After all microinjections of forskolin, 2 hr recordings were made 24 hr (n=17) and 48 hr (n=17) post-injection and 24 hr (n=12) after dibutyryl cAMP. The carbachol-induced REM sleep-like state was significantly ($p<0.05$) decreased by mPRF pretreatment with forskolin (-34.7%) and dibutyryl cAMP (-49.5%). In contrast, forskolin alone and dibutyryl cAMP alone had no immediate or long term effect on natural REM sleep. These data suggest that cholinergic REM sleep generation is mediated, in part, by pontine adenylate cyclase and cAMP.
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813.3

GONADAL STEROID MODIFICATIONS OF RECEPTOR-INDUCED RESPONSES IN CULTURED NEURONAL CELLS. I. López-Coviella, R. Alonso*, & F. Hernández-Díaz. Lab. of Neuroendocrinology, University of La Laguna Medical School, 38320 S/C de Tenerife, Spain.

The generation of second messengers following receptor activation may depend on receptor sensitivity, coupling of agonist-bound receptor to GTP-binding proteins, and extent of activation of effector proteins. Steroid hormones may affect each one of these steps. Incubation of GnRH producing neurons (GT1-7 cell line) for 48 hours with 100 nM estradiol (E_2) completely suppressed norepinephrine-induced intracellular cAMP accumulation. This effect was completely reversed by a 15-minute incubation period with 100 nM progesterone (Pg) or progesterone-3-BSA (Pg-BSA; a derivative that does not cross cell membranes), prior to norepinephrine (NE) treatment. The addition of 1 μM colchicine to the medium of E_2-untreated cells, 1 hour before harvesting, produced a 35% increase in basal cAMP levels. This increment did not occurred in E_2-treated cells. However, colchicine increased cAMP levels in the presence of E_2, when cells had been stimulated with NE. Thus, as Pg and Pg-BSA, colchicine also reversed the effect of E_2. These results may constitute the first indication of a direct effect of ovarian steroids on the response of GnRH neurons to a neurotransmitter, and suggest that genomic and non-genomic effects of gonadal steroids could alter receptor-effector coupling by different mechanisms. Data on steroid receptors and cytoskeletal proteins that may be regulating these events in this and other cell lines are presented. (Supported by DGICYT PM92-0160, GAC 92-069, & GAC 93-002)

813.4

CHRONIC ANTIDEPRESSANTS TREATMENT ENHANCE ROLIPRAM INDUCED BEHAVIORS IN RATS H. Ozawa*[1], H. Kamada[1], M. Yamamoto[1], N. Amemiya[2], S. Hatta[2], T. Saito[1], H. Ohshika[2] and N. Takahata[1]. Dept. of [1]Neuropsychiatry and [2]Pharmacology, Sapporo Medical University, School of Medicine, Sapporo 060, Japan.

It has been reported that treatment with rolipram, a selective cAMP phosphodiesterase inhibitor which reflects the enhanced availability of cAMP in the brain, results in an extraordinary behavioral syndrome characterized by head twitches (HT), forepaw shaking (FS), grooming and hypothermia in the rat. These particular behavioral alterations in rats induced by rolipram contribute to the enhanced availability of cerebral cAMP. The goal of the present study is to examine the connection between the second messenger system and animal behaviors induced by psychotherapeutic drugs. Sprague-dawley male rats were injected (i.p) once daily for 1-21 days with saline and amitriptyline (10mg/Kg), imipramine (10mg/Kg), clomipramine (10mg/Kg), milnacipran (20mg/Kg), fluoxetin (10mg/Kg), fluvoxamine (25mg/Kg), trazodone (20mg/Kg), chlorpromazine (10mg/Kg) or lithium (3mEq/kg). The numbers of rolipram (10 mg/Kg) induced HT, FS and Gr actions were counted 15min after rolipram injection for 45 min. Rectal temperature was measured immediately before and 50 min. after rolipram administration. Administration of chronic (14-21days) but not acute tricyclic antidepressants and atypical (trazodone) or novel antidepressants (SSRIs or milnacipran) augmented rolipram-induced behaviors (especially HT). Contrary to the effects of antidepressants, chronic chlorpromazine or lithium administration decreased those behaviors. These findings provide symptomatic documentation that elevation of the cAMP cascade system may have an important role in antidepressive effects .

813.5

FUNCTIONAL CHARACTERIZATION OF A2b ADENOSINE RECEPTORS EXPRESSED BY HUMAN NONPIGMENTED EPITHELIAL (NPE) CELLS *S. Mehdi Rezazadeh*, Felicia Terry and Michael W. Martin*. Department of Pharmacology, University of North Texas Health Science Center at Forth Worth, Fort Worth, Texas 76107.

Activation of A1 and A2 adenosine (ADO) receptors results in either inhibition or stimulation of adenylyl cyclase (AC), respectively. In the present study, we characterized the AC responses of human NPE cells to a series of ADO receptor agonists and antagonists. Accumulation of cyclic AMP (cAMP) was determined by measuring the conversion of ^{3}H-ATP to ^{3}H-cAMP after pre-labelling cells with ^{3}H-adenine. Cells incubated in serum-free media had low levels of cAMP and the PDE inhibitor RO20-1724 increased these levels only marginally. Addition of the isoproterenol (ISO) or the receptor-independent activator forskolin (FSK) markedly elevated cAMP levels. No evidence for A1 receptor-mediated inhibition of basal, ISO-, or FSK-stimulated AC activity was observed. In fact, the A1-selective agonist 6-chloro-N^6-cyclopentyladenosine stimulated AC activity at concentrations >10 μM and in the presence of 1 μM FSK synergistically activated cAMP accumulation, even at nanomolar concentrations. Adenosine stimulated cAMP accumulation but with relatively low potency (EC_{50} = 54 μM). The rank order of potency of other receptor agonists was NECA > R(-)PIA > CGS21680. 5'-N-ethylcarboxamidoadenosine (NECA)-stimulated (1μM) cAMP accumulation was competitively antagonized by both xanthine (8-cyclopentyl-1,3-dipropylxanthine [CPX] and 8-(3-chlorostyryl)caffeine [CSC]) and non-xanthine (CGS 15943A) ADO antagonists. The rank order of potency was CSC>CGS 15943>CPX. These data indicate that NPE cells express a "low affinity" A2b receptor subtype. (Supported by NIAAA-A06890 and UNTHSC Grants).

813.6

PK 11195 BLOCKS THE INHIBITORY EFFECT OF BENZODIAZEPINES ON FORSKOLIN-STIMULATED ADENYLATE CYCLASE ACTIVITY IN STRIATUM. C.C. Tenn*, J.M. Neu and L.P. Niles. Department of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

In this study, we investigated the effects of benzodiazepine (BZ) receptor antagonists, on the inhibition of forskolin-stimulated adenylate cyclase (AC) activity by various BZs in the rat striatum. Male Sprague Dawley rats (200-250g) were maintained under a 12L:12D lighting regimen and sacrificed approximately 3 hours after lights on. Saturation studies using [^{3}H]flunitrazepam revealed a single binding site in the striatum (K_D = 5.1 ± 0.3 nM) which significantly decreased in affinity in the presence of GTP (K_D = 10.2 ± 1.0 nM) with no change in B_{MAX}. Functional studies in the striatum revealed a biphasic inhibition of forskolin-stimulated AC activity by the peripheral type agonist, Ro5-4864. The first phase of the AC inhibition is consistent with a G_i-coupled receptor-mediated action, whereas the second phase appears to involve a direct effect on the enzyme itself. The first phase of the inhibition (10^{-11}- 10^{-5} M) had an EC_{50} of 66 nM, with the second phase (10^{-4}-5 x 10^{-4} M), an EC_{50} of 3.2 x 10^{-4} M. In the presence of flumazenil (1 μM), a central-type BZ receptor antagonist, a shift to the left was observed with the first phase of inhibition (EC_{50} = 46nM) with no significant change in the second phase.

In the presence of 5 μM of PK 11195 (a peripheral-type BZ receptor antagonist), Ro5-4864-induced inhibition of the AC activity of the first phase was reversed with no significant effect on the second phase. The peripheral antagonist similarly blocked the inhibitory effect of other BZs (flunitrazepam and diazepam) and indoleamines (melatonin and 2-iodomelatonin) on stimulated AC activity. These results provide further evidence of a G_i-coupled BZ receptor in the rat striatum. (Supported by a NSERC Strategic grant).

813.7

MODULATION OF STRIATAL *C-FOS* EXPRESSION BY DIAZEPAM. L.P. Niles*, L.J. Smith and C.C. Tenn. Department of Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Diazepam, a non-selective benzodiazepine (BZ) receptor agonist, inhibits cyclic AMP (cAMP) production, particularly in the rat striatum, via a putative G_i-coupled BZ receptor. Since phosphorylation of a cAMP response element binding protein (CREB), by a cAMP-dependent protein kinase (PKA), results in *c-fos* activation, the effect of diazepam on the expression of this immediate-early gene was examined. Male Sprague Dawley rats (about 150-250 g) were maintained for at least one week under a 12L:12D lighting cycle with lights on from 7 am to 7 pm. They were randomly assigned to groups and injected with vehicle or drugs 4 - 5 hours after lights on. Two hours later, animals were killed and striata dissected on ice and frozen overnight at -20° C. Subsequently, striata were homogenized in sucrose (containing Mg^{2+} and Hepes) and nuclear fractions isolated by centrifugation. Proteins were separated by SDS/PAGE followed by Western blot analysis using a polyclonal anti-c-FOS antibody. Following incubation with an HRP-conjugated second antibody, FOS and related proteins were detected by chemiluminescent autoradiography.

Preliminary studies indicate that diazepam (5 mg/kg, ip) induces *c-fos* expression when administered alone. However, pretreatment with diazepam (5 mg/kg, ip) caused a significant attenuation of the effect of amphetamine (5 mg/kg, ip), which by itself is a potent *c-fos* inducer. The sites and mechanisms underlying the modulatory effects of diazepam on neuronal activation, as indicated by *c-fos* induction in the striatum, await clarification. (Supported by a NSERC Strategic grant).

813.8

IN VIVO INHIBITION OF CA^{2+}/CALMODULIN STIMULATION OF TYPE I ADENYLYL CYCLASE BY SOMATOSTATIN. Mark D. Nielsen, Enrique C. Villacres*, and Daniel R. Storm, Department of Pharmacology, University of Washington, Seattle, WA 98195

In vitro evidence has demonstrated that the type I Ca^{2+}/calmodulin-sensitive adenylyl cyclase (I-AC) is inhibited by both the α_i and $\beta\gamma$ subunits of guanine nucleotide-binding proteins (G proteins). However, *in vivo* evidence concerning the ability of extracellular hormones to inhibit the Ca^{2+}/calmodulin stimulation of I-AC is lacking. Here, we describe concentration-dependent inhibition of Ca^{2+}/calmodulin stimulation of I-AC *in vivo* by the tetradecapeptide hormone somatostatin. Treatment of human embryonic kidney 293 (HEK 293) cells stably expressing I-AC with the muscarinic agonist carbachol or the Ca^{2+} ionophore A23187 resulted in 3-or 20-fold increases in intracellular cAMP levels, respectively. Utilizing the endogenous somatostatin receptors expressed in HEK 293 cells, we found that somatostatin inhibited these cAMP increases 40-50%. Forskolin stimulation of I-AC was also inhibited by a similar amount. Maximal inhibition of I-AC occurred at approximately 200nM somatostatin, with an IC50 of ~25nM. Additionally, inhibiton of Ca^{2+}/calmodulin stimulation of I-AC was prevented by overnight pretreatment with pertussis toxin, suggesting that G proteins of the G_i/G_O class are involved. These results demonstrate that extracellular hormones are capable of inhibiting Ca^{2+}/calmodulin stimulation of I-AC via inhibitory G proteins *in vivo*.

813.9

cAMP-DEPENDENT PROTEIN KINASE A INCREASES THE EXCITABILITY OF EXPIRATORY NEURONS OF CAT. O.Pierrefiche, A.M. Bischoff, P.M. Lalley* and D.W. Richter. II. Institute of Physiology, University of Göttingen, FRG and Department of Physiology, University of Wisconsin, Madison, USA.

The cAMP/PKA system is known to control neuronal excitability by altering neurotransmitter- and voltage-controlled conductances. In this study, we set out to determine whether the cAMP-PKA system modulates the excitability of late expiratory (E2) neurons in the caudal brainstem. We injected wiptide, an inhibitor of PKA, into E2 neurons in pentobarbital-anesthetized, paralyzed and artificially ventilated adult cats, and analyzed various cellular properties in single electrode current (SECC) and voltage (SEVC) clamp. Wiptide reduced neuronal input resistance, hyperpolarized the membrane potential, reduced rate of depolarization to threshold and depressed action potential discharge. Action potential duration was reduced whereas their after-hyperpolarizations increased in magnitude and duration. Inspiratory- and postinspiratory-phased synaptic outward currents increased greatly and expiratory-phased synaptic inward currents seemed slightly depressed.

The results indicate that the cAMP/PKA system is tonically active and increases excitability of E2 neurons. Possible mechanisms for these effects include blockade of persistant potassium and GABA-A receptor-controlled currents, as well as augmentation of glutamate receptor-controlled currents

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813.10

ACETYLCHOLINESTERASE MODULATES DOPAMINE - STIMULATED CYCLIC AMP ACCUMULATION IN *APLYSIA* GILL.

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Acetylcholinesterase (AChE) has functions in addition to acetylcholine (ACh) hydrolysis as supported by studies of AChE regulating dopaminergic receptor responsiveness in substantia nigra (Greenfield, S.A., 1985). Our recent studies have demonstrated that AChE inhibition affects a non-cholinergic component of the siphon-gill withdrawal reflex in *Aplysia*. Dopamine (DA), enhances muscle contraction in gill apparently by elevating cyclic AMP (cAMP) levels (Ruben et al., 1979). AChE is widely present in *Aplysia* gill and is released when gill is exposed to DA suggesting a non-cholinergic functional interaction between DA and AChE in the gill. The aim of the present study was to determine if AChE inhibition in the gill alters DA-dependent cAMP accumulation, as measured by RIA. DA dose-dependently increased cAMP levels in isolated gill pinnules incubated in *Aplysia* saline. Incubation in the presence of AChE inhibitor BW284c51, which binds to both the catalytic site and a peripheral anionic site of AChE, dose-dependently attenuated cAMP accumulation in response to a maximum stimulatory dose (30μM) of DA. Total inhibition of AChE with 100μm BW284c51 reduced DA-dependent cAMP by 68%. In contrast, total inhibition of AChE with 1mM edrophonium chloride, which binds only to the catalytic site of AChE, did not reduce DA stimulated cAMP levels. Also, incubating in carbachol (10 and 100μM) had no effect on cAMP levels in pinnules. These results show that AChE modulates DA-dependent cAMP accumulation in *Aplysia* gill through a non-cholinergic mechanism involving its peripheral site. AChE's modulation of DA's effects in *Aplysia* gill and nigral neurons in higher vertebrates suggests that this function of AChE is conserved across species.

813.11

cAMP IMAGING REVEALS GLUTAMATE RECEPTOR-MEDIATED REDUCTION OF [cAMP] IN PRIMARY CULTURES OF HIPPOCAMPAL NEURONS. B.J. Bacskai[1] and R.Y. Tsien*[1,2]. [1]Dept. of Pharmacology and [2]Howard Hughes Medical Institute, University of California San Diego, La Jolla, CA 92093-0647.

The role of cAMP in neuronal plasticity in the hippocampus is receiving considerable attention. However, traditional radio-immunoassays measure total cAMP at a single time point, usually in the presence of phosphodiesterase inhibitors, in homogenates of brain which contain neurons, astroglia, and other cell types. Using a fluorescently labeled cAMP-dependent protein kinase (FlCRhR), cytosolic [cAMP] was measured in morphologically identified neurons in primary cultures of rat hippocampus in real time. Individual neurons were microinjected with FlCRhR and stimulated with a variety of glutamate receptor agonists. Resting [cAMP] was high enough to cause partial activation of the kinase, and rapidly decreased to negligible levels following a 1-5 min pulse of 10-100 μM glutamate. NMDA (100 μM) and AMPA (100 μM) also elicited decreases in [cAMP]. Ca^{2+} entry seemed necessary but not sufficient, because reductions in [cAMP] required extracellular Ca^{2+}, and nitrendipine blocked the reduction in [cAMP] induced by AMPA, but not that by NMDA. However elevation of Ca^{2+} by high K^+ or ionomycin had no effect. Forskolin (25 μM), which normally elicited maximal increases in [cAMP], had no effect when applied within 20-30 minutes after glutamate or NMDA addition. The metabotropic glutamate receptor agonist trans-ACPD had no effect on basal or forskolin-stimulated [cAMP]. Bath application of glutamate or NMDA led to rapid, transient cytosolic acidification from pH 7.0 to pH 6.5, in the range where FlCRhR fluorescence is partially sensitive to pH, but acidification alone cannot completely account for the measured decreases in FlCRhR ratio, nor the inhibition of forskolin-stimulated increases in [cAMP]. Inhibitory crosstalk between glutamate receptors and cAMP may be important for neuronal plasticity.

813.12

ENDOGENOUS PROTEIN KINASE A INHIBITOR (PKIα) MODULATES SYNAPTIC ACTIVITY

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Protein kinase A (PKA) has long been known to be involved in major regulatory mechanisms underlying synaptic plasticity and complex behaviors such as learning and memory. PKIα has been extensively used as an extremely specific and potent inhibitor of PKA and the PKA-mediated signal transduction. Clear functions 'in vivo' for PKIα, however, remain to be established. Here we report that several forms of synaptic stimulation in the rat hippocampus cause a 50% decrease in the concentration of PKIα. Furthermore, infusion of antisense oligonucleotides against PKIα into the rat brain results in dramatic changes in the electrophysiology of hippocampal neurons, including the blocking of long-term potentiation suggesting a stimulus-dependent regulatory role for PKIα in PKA signal transduction.

813.13

PHOSPHORYLATION OF DARPP-32 AND INHIBITOR-1 BY CYCLIN-DEPENDENT PROTEIN KINASES. J.A. Bibb[1], J.P. O'Callaghan[1], S.L. Pelech[2], J.-A. Girault[3], A.C. Nairn[1]*, J. H. Wang[4], P. Greengard[1] and A.J. Czernik[1]. [1]Lab. of Molecular & Cellular Neuroscience, The Rockefeller University, New York, NY 10021; [2]University of British Columbia, Vancouver, Canada; [3]INSERM U114, Paris, France; University of Calgary, Calgary, Canada.

DARPP-32 (D-32) and inhibitor 1 (I-1) are homologous inhibitors of protein phosphatase 1 (PP-1). Their ability to function as inhibitors is dependent on phosphorylation of threonine 34 and threonine 35 of D-32 and I-1, respectively, by cAMP-dependent protein kinase. For D-32, which is highly enriched in dopaminoceptive neurons of the basal ganglia, this activated state is modulated through phosphorylation at additional sites by casein kinases I and II. Here, we report that *in vitro*, D-32 and I-1 serve as substrates for phosphorylation by the cell-cycle dependent kinase cdc 2 as well as the neuronal cdc 2-like kinase cdk 5. The stoichiometries of phosphorylation by cdc 2 purified from sea star oocytes have been determined for D-32 and I-1, respectively. Two-dimensional phosphopeptide mapping of thermolytic and tryptic digests of D-32 and I-1 revealed distinct maps for each. Amino acid analysis identified the residues phosphorylated as threonine for D-32 and serine for I-1. Sequence analysis of phosphopeptides purified by HPLC was used to identify the phosphorylation sites and direct the generation of phosphorylation-state-specific antibodies. The antibodies are being used to evaluate the state of phosphorylation of these sites in tissue slices. Neuronal cdk 5 purified from bovine brain appears to phosphorylate the same site as cdc 2 based on phosphopeptide mapping. The expression of various cdks in striatal tissue was shown to be developmentally regulated by immunoblot analysis. Notably, of the kinases studied, only cdk 5 was detected in adult rat striatal tissue.

813.14

EXPRESSION OF PKI ISOFORM mRNAS IN DEVELOPING RAT NERVOUS TISSUE; LOCALISATION OF mRNA SPECIES IN BRAIN. L. F. Donaldson*, M. R. Hanley and D. A. Walsh Dept. of Biological Chemistry, UCDavis, Davis Ca 95616

The mRNAs for the two known isoforms of the cAMP-dependent protein kinase (PKA) inhibitor protein (PKIα and PKIβ) are known to have a differential distribution in developing rat tissues, particularly in brain and testis. As these tissues are heterogenous in cell type, we have investigated the cellular expression of PKIα and PKIβ mRNAs in developing rat brains using a specific in situ hybridisation technique. Male Wistar rats were sacrificed at 5 day intervals, beginning at day 5 of age and ending at day 60. Whole brains were removed, hemisected along the sagittal plane and rapidly frozen on dry ice. 10μm sections were hybridised with cRNA probes complementary to the mRNAs encoding PKIα and PKIβ. Film autoradiography showed high expression of PKI mRNAs in cerebellum and hippocampus from days 15 to 60. In immature rats (d5) PKIβ mRNA expression was high in individual cortical and hippocampal neurons. In adults PKIβ mRNA was appparent in cortical, hippocampal, and cerebellar granular neurons. This expression was detectable from day 15, increased to a plateau by day 25 and remained constant until day 60. No specific hybridisation was detectable in cerebellum or hippocampus before 20 days, or in sections hybridised with sense cRNA probes. PKIα was also localised in cerebellar granule cells and hippocampus in adult rat brain. PKA has been shown to be of importance in the maintenance of long term potentiation and response of hippocampal neurons to anoxia, suggesting that PKI may play an important role in the function of the hippocampus.

813.15

BILATERAL DOPAMINE LESIONS IN THE NUCLEUS ACCUMBENS MODIFIES CYCLIC AMP-DEPENDENT PHOSPHOPROTEINS. L. Churchill*, Kari J. Johnson and P.W. Kalivas. Dept. of Veterinary & Comparative Anatomy, Pharmacol. & Physiol. Washington State University, Pullman, WA 99164-6520.

Previous analyses revealed that cyclic-AMP dependent back phosphorylation of a 35 kD nucleus accumbens protein was reduced at 32 days after bilateral 6-hydroxydopamine lesions in the nucleus accumbens. Two-dimensional gel electrophoretic analyses verified that a 35 and a 56 kD protein showed a significant decrease in cyclic AMP-dependent phosphorylation *in vitro*. Also a protein with a molecular weight of 45 kD showed a significant enhancement in the dopamine-depleted nucleus accumbens. These results indicate an increase in the phosphoproteins with molecular weights of 35 and 56 kD as well as a decrease in the phosphoprotein at 45 kD after a bilateral dopamine lesion. The phosphoprotein of 35 kD might be DARPP-32 as evidenced by the changes in phosphorylation that occurred 17 days after a unilateral dopamine lesion in the striatum with dopamine priming (Barone et al., 1994, *Neuroscience* 61:867). The differences between this study and ours may be due to differences between the nucleus accumbens and striatum, the longer time period studied, or the bilateral vs. unilateral lesion. A further question is whether the μ-opioid agonist induction of behavioral augmentation after the dopamine lesion will also alter phosphoproteins in the nucleus accumbens. This work was supported in part by USPHS grants #DA06612 (LC) and #MH40817, #DA0396, #DA00158 (PWK).

813.16

ISOLATION AND CHARACTERIZATION OF cAMP-SPECIFIC, ROLIPRAM-SENSITIVE PHOSPHODIESTERASE (PDE IV) FROM PIG BRAIN CYTOSOL. Gudrun Pahlke and Herbert H. Schneider* Research Laboratories, Schering AG, 13342 Berlin, Germany.

Rolipram is a selective and stereospecific inhibitor of type IV PDE. Using a rapid and specific purification procedure we have purified the cytosolic form of a rolipram sensitive "low K_m" cAMP PDE from pig brain cortex. The enzyme was isolated by affinity batch chromatography followed by MonoQ and Superose6 chromatography. The purification was approx. 5000-fold at 1% yield based on ^{3}H-rolipram binding capacity. The protein had a native MW of 180 kDa and a denatured MW of 90 kDa, suggesting a dimeric structure. K_D (0.2 nM) and n_H near 1 of ^{3}H-rolipram binding were similar throughout purification steps in the presence of a factor derived from boiled tissue extract. In its absence B_{max} decreased drastically with unchanged K_D and n_H. It is a thermostable, lipophilic and Mg^{++}-dependent protein which modulates the binding of ^{3}H-rolipram to the protein in a positive or negative manner, depending on concentration. The sensitivity of the PDE activity to inhibition by (-)-rolipram improved from 300 nM (IC_{50}) in crude brain extract to 0.7nM in the purified preparation. It decreased further to 0.15nM in the presence of factor. IBMX, ICI 63.197, Ro 20-1724 and denbufylline were active with IC_{50}s of 740, 140, 33 and 5nM. Cilostamide inhibited enzyme activity significantly only at 100μM. Between 0.1-100μM, cGMP neither inhibited nor activated the purified enzyme. Calmodulin stimulated PDE activity in crude extracts 2-fold, no effect was seen in MonoQ fractions. The isolated protein revealed complex enzyme kinetic behavior. Depending on substrate concentration K_m and V_{max} values varied between 2.8 and 7.3 μM and V_{max} between 13 and 26 μmoles/min/mg. The type of enzyme inhibition by (-)-rolipram was of a hyperbolic mixed type, probably because of the endogenous mixture of PDE IV isoenzymes.

814.1

ALPHA-IFN MODULATES THE RESPONSE TO GABA AND GLUTAMATE ON NEURONS FROM LATERAL HYPOTHALAMUS. V. Mendoza-Fernández, P. Pérez-Arellano, M. Herrera-Ruíz and C. Reyes-Vázquez*. Depto. Fisiol. Fac. Med., UNAM, México, D.F., México

Both, systemic and microiontophoretic application of α-interferon (α-IFN) exerts long lasting effects on the electrical activity of several brain nuclei; particularly, the lateral hypothalamic nuclei had shown to be very sensitive to the effects of this cytokine. In these neurons α-ifn elicits changes in the pattern, size and frequency of discharge, of the electrical signals recorded extracellularly. These effects started few seconds after α-IFN application and always outlasted the administration for several min. However, the relation between this effect and the physiologic response to other substances, is unknown. In the present study we analyzed the effects of GABA and glutamate, applied before and after an α-IFN administration, on the electrical activity of lateral hypothalamic neurons. The recordings were made from brain slices obtained from male Wistar rats. Slices preparation and recording techniques of the extracellular activity were made using conventional methods. Alpha-IFN elicited effects in 82% of the recorded neurons. Approximately 64% of these neurons showed a decrease of their discharge with changes in the size and the pattern of the action potentials. When applied before α-IFN administration, GABA and Glutamate showed a dose dependent decrease and increase; respectively, of the frequency of discharge. Such effects lasted exclusively the time of drug application, and once it ended, the frequency of discharge returned to control levels without changes in the size or the pattern of response. However, when these drugs were applied after α-IFN administration, the effects were increased importantly, both, in intensity and duration. This potentiation action started between 10 and 15 min after α-IFN application and lasted several hours. After that, the sensitivity of these neurons to these drugs returned to normal. The results show that α-IFN modifies the sensitivity and response pattern to other substances from hypothalamic neurons.

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814.2

MODULATION OF NMDA RESPONSES BY INTERFERON-α IN THE PREOPTIC ANTERIOR HYPOTHALAMUS. S.Take, T.Katafuchi, S.Duan and T.Hori. Dept of Physiology, Faculty of Medicine, Kyushu University, Fukuoka 812-82, Japan

The brain and the immune system share the ligand-receptor systems which enable them to communicate with each other. Interferon-α (IFN-α) is one of such ligands that are produced in the both systems, which is known to induce a variety of central symptoms. The preoptic anterior hypothalamus (POA) is one of the sites of action of central IFN-α. We previously reported that IFN-α's modulatory effect on glutamate- and NMDA-induced whole cell currents in the POA. In the present study, we investigated the mechanisms of IFN-α-induced suppression of NMDA responses in the POA neurons voltage-clamped at -60mV using 120μm hypothalamic slices of 10-20 days old WKA rat. Inward currents induced by pressure-ejected NMDA (200μM, 200msec) were markedly suppressed by perfusion of recombinant human IFN-α (100-200U/ml, 2 min) with a few minutes latency for more than one hour. This IFN-α-induced suppression of NMDA currents was partially blocked by simultaneous application of naloxone (10μM), and the bath application of [Tyr-(D-Ala)-Gly-(N-Me-Phe)-Gly-ol]-enkephalin (DAGO, 2μM), μ receptor selective opioid agonist, suppressed NMDA currents just as IFN-α did, suggesting the involvement of opioid receptors. Although bath application of sodium salicylate (SALC, 10μM) reversed suppression of NMDA currents, prostaglandin E2 (2μM) did not affect NMDA responses. Concurrent application of superoxide dismutase (SOD, 200U/ml) with IFN-α almost completely abolished IFN-α's action on NMDA currents and hydrogen peroxide (1mM) mimicked it, suggesting the involvement of reactive oxygen intermediates (ROIs). Neither SOD nor SALC affected DAGO-induced suppression of NMDA currents. Nω-nitro-L-arginin (100μM) slightly attenuated the suppression of NMDA currents, suggesting the involvement of nitric oxide which is known to interact with ROIs.

These results suggest that opioid receptor mechanism and ROIs are independently involved in IFN-α-induced suppression of NMDA currents in the POA neurons.

814.3

PROLONGED STIMULATION WITH INTERFERON-α REGULATES CATECHOLAMINE SECRETION IN CULTURED BOVINE ADRENAL CHROMAFFIN CELLS. Y.Toyohira, N.Yanagihara, K.Minami[1], Y.Uezono and F.Izumi*. Depts of Pharmacology and [1]Anesthesiology, Univ. of Occup. & Envir. Health, School of Medicine, Kitakyushu 807, Japan.

Interferons(IFNs) are a group of molecules that have antiviral and immunoregulatory functions. Recent studies have reported that several neurological side effects are observed in IFN-treated patients. To investigate whether IFN can modulate the neurosecretory functions, the effects of two IFNs, human leukocyte IFN-α and recombinant human IFN-γ, were studied in cultured bovine adrenal chromaffin cells. Treatment of cultured cells with IFN-α(1000U/ml) for 48 hr increased an accumulation of catecholamines in the cultured medium, but not with IFN-γ(1000U/ml). The IFN-α-induced response was observed in time (8-48 hr)- and concentration (10-1000 U/ml)-dependent manners. The stimulatory effect of IFN-α was not inhibited by protein kinase C inhibitor (H-7) and NO synthase inhibitor (L-NMMA), both of them were previously reported as the second messenger in catecholamine secretion. These results suggest a possibility that IFN-α acts as a neuromodulator during immune responses.

814.4

AVP release by acetylcholine or norepinephrine: region-specific and cytokine-specific regulation. J.Raber*, G.F.Koob, and F.E.Bloom, Department of Neuropharmacology, The Scripps Research Institute, 10666 North Torrey Pines Rd., La Jolla, CA 92037.

Arginine vasopressin (AVP) containing neurons of the magnocellular hypothalamic nuclei and limbic structures, including the hippocampus and amygdala may be influenced by cytokines. Interferon-α (IFN-α) and transforming growth factor-β1 (TGF-β1) have been detected in the brain, suggesting their possible regulatory functions. An *in vitro* paradigm was used to investigate the influence of these cytokines on the release mechanisms of AVP in the hypothalamus and amygdala. Previous work established that *in vitro* AVP is released, in a calcium-dependent manner from both sites, and that this release is responsive to acetylcholine (ACh), norepinephrine (NE), IL-1, IL-2, IL-6 or high KCL (60 mM). IFN-α enhanced AVP and CRF release from both hypothalamus and amygdala. Dibutyryl cGMP also stimulated AVP release. Blockade of nitric oxide synthase antagonized the IFN-α-induced AVP release from the amygdala but not from the hypothalamus. TGF-β1 had no effect on basal release of AVP, nor on the AVP-release induced by IFN-α, IL-2 or NE, but selectively blocked the ACh-induced release in both hypothalamus and amygdala. TGF-β1 also inhibited the ACh-induced CRF release from both brain regions. When the release of AVP induced by IFN-α, IL-2, ACh and NE was probed with inhibitors of guanylate cyclase, the interactions exhibited regional selectivity: neither the IL-2-induced AVP release from hypothalamus, nor the NE-induced release of AVP from either amygdala or hypothalamus were affected by guanylate cyclase inhibitors, but all other AVP releasers were blocked. These data suggest that in addition to CRF, AVP release by IFN-α may also contribute to the activation of the hypothalamic pituitary axis. The ability of TGF-β1 to diminish the AVP and CRF released by ACh could mediate some of this cytokine's capacity to rescue damaged neurons. The extension of these neurotransmitter-cytokine interactions to the amygdala may provide an additional basis for interactions between neuronal and immune systems. (supported by grant MH 47680).

814.5

LOCAL LYMPH NODE APPLICATION OF 6-HYDROXYDOPAMINE (6-OHDA) DENERVATES LYMPHOID ORGANS WHILE SPARING INNERVATION TO THE JOINTS: A MODEL FOR STUDYING NEURAL-IMMUNE INTERACTIONS IN EXPERIMENTAL ARTHRITIS (EA). D. Lorton*, D.L. Bellinger, S.Y. Felten, and D.L. Felten. Hoover Arthritis Research Center, Sun Health Research Institute, Sun City, AZ 85351 and Dept. of Neurobiol. and Anat., Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Numerous studies document that NE can modulate chronic inflammatory responses and joint destruction associated with rheumatoid arthritis (RA). These studies have employed systemic methods of NA denervation which denervate several potential sites where NE may act, including the joints and lymphoid organs. We propose that NA innervation is playing a dual role: modulating the severity of RA by innervation of 1) the joints and 2) the lymphoid organs. We have developed a model to explore the role of NA innervation of lymphoid organs in the development of EA.

The fatpads that sequester the popliteal and inguinal lymph nodes (PLN and ILN, respectively) were isolated on each side of eight male Lewis rats, and two 4 μl injections of 6-OHDA (150 μg/ml sterile saline plus 0.1% ascorbic acid) or vehicle were administered 10 minutes apart into each fatpad. Lymph nodes (LN), spleen, sciatic nerve and heart were taken 1, 5, and 14 days following localized 6-OHDA injection and NE content was determined using HPLC to quantitate levels of NE. By one day following local 6-OHDA application into ILN and PLN, NE levels were reduced by approximately 87% and 82% compared to control values, respectively. Reduced ILN and PLN NE levels persisted over the fourteen day time period. In contrast, LN application of 6-OHDA did not significantly reduce NE concentration in the sciatic nerve. The local 6-OHDA injection did, however, result in a significant reduction of NE content in the spleen and heart over fourteen days. Thus, local ILN and PLN application of 6-OHDA reduced NE levels in the relevant LN and spleen, and spared the NE innervation of the hindlimbs. This method of denervation will be a useful model for dissecting out the functional role of NA innervation of lymphoid organs versus the joints in the onset and progression of EA.

814.6

SYMPATHETIC NORADRENERGIC INNERVATION OF SPLEEN, THYMUS AND MESENTERIC LYMPH NODES (MLN): A COMPARISON BETWEEN SPRAGUE-DAWLEY, LEWIS AND FISCHER 344 RATS. S.S. Dimitrova, S.Y. Felten and D.L. Felten*. Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Primary and secondary lymphoid organs are innervated by sympathetic postganglionic noradrenergic (NA) nerve fibers. We have shown that the NA nerves in the spleens of Fischer 344 (F344) rats progressively decline with age, paralleled by an age-related decline in cell-mediated immunity. The histocompatible inbred Lewis (LEW) strain is susceptible to autoimmune disorders and shows exacerbated autoimmune reactivity following sympathectomy. This study was undertaken to compare the NA innervation of spleen, thymus and MLNs in young adult, male and female, F344, LEW and Sprague-Dawley (SD) rats, using anatomical and neurochemical assessment. Both strain and sex effects were observed in splenic innervation. LEW rats have higher NE concentration than both SD and F344 rats. NE concentration is higher in females of all strains compared to males. The F344 strain has lower total NE content in spleen than each of the other two strains, but no male/female differences were noted. The distribution of NA nerve fibers in the spleens of the three strains was similar and followed the compartmentation reported previously. The density of innervation correlated well with the neurochemical analysis (SD>LEW>F344). In the thymus, total NE was greater in LEW than F344 rats. No sex differences were found. In the MLN, the NE concentration in the SD strain is higher than in F344; for total NE content, SD>LEW>F344.

This study demonstrates that male F344 rats have the most sparsely innervated spleens, the lowest NE concentration in spleen and MLN, and the lowest NE content in the lymphoid organs examined. This observation may be important for interpreting aging studies in rodents, where the male F344 rat is the single best available model at present. The more dense NE innervation of secondary lymphoid organs in LEW rats may be important in the regulation of autoimmune reactivity. (Supported by R37 MH 42076 and a Markey Charitable Trust Award).

814.7

NGF MODULATES SYMPATHETIC INNERVATION OF LYMPHOID TISSUES. Sonia L. Carlson*, Kathryn M. Albers[§], Daniel J. Beiting, Mark Parish, James M. Conner[†], & Brian M. Davis. Dept. of Anatomy and Neurobiology & [§]Dept. of Pathology, Univ. of Kentucky College of Medicine, Lexington, KY 40536-0084 and [†]Dept. of Biology, Univ. of California, San Diego, La Jolla, CA 92093-0601

Immune tissues are known to be innervated by the sympathetic nervous system, but little is known of what directs the innervation to specific tissue compartments. We have examined the sympathetic innervation of immune tissues in transgenic mice that overexpress nerve growth factor (NGF) in skin and other epithelial structures. NGF transgenic mice exhibited dramatic hyperinnervation in the splenic marginal zone, and the medulla and capsule of peripheral lymph nodes. In contrast, the transgenic mesenteric lymph nodes showed no hyperinnervation. This difference correlated with the location of these nodes; peripheral lymph nodes drain skin where the transgene was expressed while mesenteric lymph nodes drain non-transgene expressing structures. In addition, the level of innervation correlated with the level of NGF peptide content as assayed by ELISA and immunocytochemistry. RT-PCR showed that the NGF transgene was not being expressed in the immune tissues, suggesting that immune tissues can concentrate transgene-produced NGF. In addition, the change in innervation had functional consequences. The mitogen response to concanavalin A (Con A) by spleen cells was decreased in the transgenics suggesting that elevated catecholamines or NGF can modulate the proliferative response of these cells. These mice demonstrate that NGF can modulate the sympathetic innervation and function of the immune system. Supported by MH48644 to SLC and NS31826 to BMD & KMA.

814.8

ROLE OF PROTEOLYTIC ENZYMES AND PLASMA INHIBITORS IN CONTROLLING OPIOID PEPTIDES-MEDIATED IMMUNOMODULATION. L. Bongiorno-Borbone*, A. Urbani, M. Marini, L. G. Roda. Dept. Medicina Sperimentale, Universita' "Tor Vergata", 00133 Roma, Italy.

Circulating immunocompetent cells release soluble proteolyses active in degrading peptides of limited molecular size, notably opioid peptides. Together with soluble plasma enzymes and with those embedded in the membrane of immunocompetent cells, these enzymes regulate the active concentration of neuromodulatory-active plasma peptides. The proteolytic activity of immunocompetent cells is controlled by stimulation: in stimulated PBMC, both the release of soluble enzymes and the enzymes exhibited by the cells' membrane are increased (Bongiorno, L., Marini, M., Urbani, A., Ausiello, C.M. & Roda, L.G. (1993) Int. J. Immunopharmac. 15:621-629). In addition, stimulation modifies the ratio between the three classes of enzymes involved in enkephalins' hydrolysis: aminopeptidases, dipeptidylaminopeptidases and dipeptidylcarboxypeptidases. The activity of all soluble plasma enzymes is controlled by the proteolysis inhibitors present in this tissue (Bolacchi, F., Marini, M., Urbani. A. & Roda, L.G. Neurochem. Res., in press). It moreover seems likely that the direction and intensity of the peptide-mediated neuroimmune interactions is modulated by the immune system activation as well. The interactions of these factors have been studied with the specific aim of ascertaining the role of proteolytic enzymes and of their inhibitors in the regulative processing of plasma peptides, specifically of opioid peptides. The role of proteases and of their inhibitors has been studied in cultured lymphoid cell lines liable to stimulation, antigen-specific clones of immunocompetent cells and human volunteers. Results obtained indicate that the variations of hydrolysis measurable under different conditions are controlled by variations of both the population of the enzymes released by immunocompetent cells and of the plasma inhibitors. These results can be interpreted as indicating that plasma inhibitors play a role in controlling the short-term variations of neuropeptides hydrolysis evidenced in response to changes in the environmental parameters (e.g. Shulteis, G. & Martinez, J.L., Jr. (1993) Peptides 14:161-167).

814.9

BOTH BRAIN AND LYMPHOCYTE DERIVED β-ENDORPHIN EXERT IMMUNOSUPPRESSION. P.Sacerdote*, B.Manfredi and A.E. Panerai. Dept.Pharmacology, University of Milan, Milano, Italy.

We previously showed that the opioid receptor antagonists naloxone and naltrexone enhance the proliferative responses of human and rat lymphocytes (1). In the present study we wanted to evaluate the role of central, pituitary and immunocyte-derived β-endorphin (BE) in the inhibitory effect of the peptide on splenocyte proliferation in the rat. The intracerebroventricular (icv) administration of BE (1,5,10 ng/rat) induced a significant inhibition of PHA induced proliferation, while the icv administration of an anti BE antiserum potentiated the response. Also the intravenous (iv) injection of the BE antiserum induced an increase of splenocyte proliferation. Since the pituitary can be considered the major source of circulating BE, in order to verify the effect of immunocyte derived BE we evaluated the effect of the administration of the i.v. antiserum in hypophysectomized rats. In these animals the BE antiserum is still able to enhance the splenocyte proliferation. Our data suggest that BE exerts a tonic inhibitory effect on proliferation acting both centrally and peripherally, where immunocyte derived BE can inhibit proliferation throughout a paracrine/autocrine mechanism.

Manfredi et al., J.Neuroimmunol. 44;43-48, 1993

814.10

ROLE OF NOREPHINEPHRINE IN ANTIBODY PRODUCTION: SUPPRESSED IgG PRODUCTION IN EPILEPSY-PRONE MICE. Julia M. Green-Johnson[1], Steve Zalcman[2], Catherine Y. Vriend, Svetlana Dolina, Dwight M. Nance and Arnold H. Greenberg. The Manitoba Institute of Cell Biology and Dept. of Pathology, University of Manitoba, Winnipeg, MB, R3E 0V9; [1]Biology Dept, Acadia University, Wolfville, NS. B0P 1X0[2] Center for Studies in Behavioural Neurobiology, Concordia University, Montreal, PQ. H3G 1M8.

To determine whether chronic neurochemical alterations could influence antibody production, the responses of two substrains of Balb/c mice [Epilepsy Prone (EP) and Epilepsy Resistant (ER)] to immunization with sheep red blood cells (SRBC) were examined. Significant differences in levels of SRBC-specific IgG were found between the two substrains. In addition, significant differences in splenic NE levels between the two substrains were observed, in both non-immunized and in immunized mice at several time points after immunization. The EP substrain showed elevated splenic NE levels and suppressed IgG production relative to the ER substrain. Treatment of ER mice with the β_2 adrenergic agonist (β_2AR) terbutaline on days 4, 5 and 6 after immunization resulted in a significant decrease in IgG production relative to saline treated controls. This suggests that elevated splenic NE acting through β_2AR is capable of mediating the suppression of the IgG response as seen in the EP strain. Taken together with previous studies indicating that the effect of elevated NE at the initiation of the immune response can have an enhancing effect on antibody production, these data suggest a dual role for NE in the immune response.

814.11

MURINE ANTI-NEURONAL IgG AUTOANTIBODIES ALTER ION CHANNEL CURRENTS OF NEUROBLASTOMA CELLS. J.Crimando, K.Cooper, and S.A.Hoffman* Ariz. St. Univ. Tempe AZ 85281.

Neurobehavioral manifestations in both human and murine models of systemic lupus erythematosus (SLE) have been correlated to diverse brain-reactive autoantibodies (BRAA). It is suggested that subsets of BRAA, especially IgG, bind to neuronal surface components and alter specific functions which may lead to some of these deficits. Using a natural murine models of SLE, we examined whether monoclonal IgG BRAA and anti-neuronal IgG autoantibodies from sera are capable of altering voltage-gated sodium (Na^+) and potassium (K^+) channel currents of neuroblastoma NBP_2 cells. Monoclonal IgG BRAA produced from a 2 mo. BXSB mouse bound brain membrane antigens of 58, 32, and 30kD, a 105kD NBP_2 membrane antigen, and reacted against neuronal cell bodies in cortex, hippocampus, and hypothalamus. Cultured NBP_2 cells were incubated with the monoclonal BRAA or anti-neuronal IgG for two hours. Inward Na^+ and outward rectifying K^+ currents were analysed by whole-cell patch-clamp recording. Though the monoclonal IgG BRAA did not affect these currents, one of the six samples of autoimmune IgG from BXSB sera did inhibit inward currents by 65% ($P<0.001$) compared to controls, while outward currents were unaffected. This demonstrates that subsets of anti-neuronal IgG exist which have specific functional effects on neuronal cells. These are hypothesized to be significant in the pathogenesis of immune-mediated neurobehavioral involvement in immunologic disorders.

814.12

THE EFFECTS OF SEX AND ESTROUS PHASE ON SURGERY-INDUCED INCREASES IN TUMOR CELL RETENTION AND ITS ATTENUATION BY MORPHINE G.G. Page*, S.A. Boun and S. Ben-Eliyahu. Ohio State Univ. College of Nursing, Columbus, OH 43210.

The vast majority of studies investigating the effects of surgery on immunity and tumor metastasis have used male subjects. We recently reported that morphine attenuates the metastatic-enhancing effects of surgery in male F344 rats. Given the differing hormonal environments between females and males and recent findings indicating that females in proestrus/estrus (PE, high estrogen levels) are more susceptible to tumor metastasis than are females in diestrus (Di), these studies explored whether there were sex- or estrous-related differences in the metastatic-enhancing effects of surgery and in the effectiveness of morphine in attenuating this outcome. A mammary adenocarcinoma cell line, MADB106, syngeneic to the F344 rat was used. MADB106 cells metastasize only in the lungs, a process known to be controlled by natural killer cells. In Exp 1, females in PE or Di and males were randomly assigned to the surgery (standard abdominal laparotomy under halothane anesthesia), anesthesia only, or control group. In Exp 2, PE or Di females and males were either subjected to abdominal surgery with anesthesia or anesthesia alone, and were either treated or not with morphine (pre- and postoperatively in saline or a slow release suspension, respectively). Radiolabeled MADB106 cells were injected i.v. 5 h after surgery and lungs were removed 13 h later to assess their radioactive content. Surgery resulted in a 3-fold increase in tumor cell retention in all 3 sex/estrous groups, an effect that was additive to the above-mentioned differences observed in the PE vs Di females in both the anesthesia only and control groups. This finding may have important implications in the timing of surgical interventions for breast cancer. Morphine administration similarly benefitted the males and females, evidenced by a significant interaction between the effects of surgery and of morphine. Supported by the Oncology Nursing Society and NIH grant NR03829.

814.13

EFFECT OF CAPSAICIN INTRAPERITONEAL ADMINISTRATION ON MESENTERIC AND PERITONEAL MAST CELLS. Y.H. Wang, J.Y. Wei and V.L.W. Go , A. Scheibel Brain Res Inst and CURE/Gastroenteric Biol Ctr, UCLA Los Angeles, CA 90024-1782.

Using video imaging and vital fluorescent probes to visualized mast cell's activation (NeuroImage 1:313-324, 1994) we have reported that electrical stimulation of splanchnic nerve activated mesenteric mast cells (MeMCs) (Neurosci Abst 20:052, 1994) and that capsaicin-sensitive splanchnic afferents is important for the action (Gastroenterology 1995, in press). Topical application of low dose capsaicin has been used to selectively excite the sensory terminals of thin primary afferents (Pharmacol Rev 43:147-148, 1991). Aim: to further determine the effect of capsaicin i.p. administration on the MeMCs and peritoneal mast cells (PMCs). Sulforhodamine B (200 μg/ml with capsaicin 5 X 10^{-5} M, 20 ml) was i.p. injected into urethane anesthetized rats and kept in situ for 20 min. Laparotomy was performed, pieces of mesentery were mounted on teflon tambours for MeMC examination and peritoneal lavage was harvested for PMC. Images of MeMCs (n=18 to 87, median 45.5 cells) were randomly captured from each tambour, and PMCs (n=50 to 138, median 72 cells) from dish. For each experiment the stained cells of two tambours and two dishes were counted, normalized and expressed as mean $\pm$ SD%. Data from three vehicle experiments were compared to three experimental trials. Results: capsaicin i.p. activated 76 $\pm$ 5.7% MeMCs as compared to 10.1 $\pm$ 4.3% N=6 vehicle trials ($p < 0.001$ two-tailed paired t test), whereas for PMCs the activated cell number is 9.7 $\pm$ 2.2% vs 10.6 $\pm$ 4.8% N=6 ($p > 0.7$ two-tailed paired t-test). The results indicated that capsaicin (5 X 10^{-5}) i.p. activated MeMCs but not PMCs, suggesting the important of neural element involvement in the effect. Neuropeptides leading to MeMC activation are further investigated. (Supported by NIH Grant NS 28433)

814.14

EXPRESSION OF NITRIC OXIDE SYNTHASE TYPE II IN THE SPINAL CORD UNDER CONDITIONS PRODUCING THERMAL HYPERALGESIA. D. Grzybicki*, A. Loihl, S. Kardos, G. Gebhart and S. Murphy. Depts. of Pharmacology and Pathology*, Univ. of Iowa, Iowa City, IA 52242.

Peripheral tissue injury or nerve damage result in a thermal hyperalgesia believed to be mediated via the production of nitric oxide (NO). Attention is on NO derived from Type I NO synthase (NOS) but sustained production of NO via the NOS II isoform may be involved in the mediation of a transient thermal hyperalgesia produced in rats after intrathecal administration of proinflammatory cytokines.

Male Sprague Dawley rats with an intrathecal sterile catheter were infused with IL-1β (10 ng) and IFN-γ (1000 U) and then sacrificed at various times. Segments of spinal cord (1 cm) centered around the lumbar enlargement were excised. Expression of NOS II mRNA was detected by RT-PCR and confirmed by ribonuclease protection assay. Western blotting revealed NOS II protein at 4 and 8 hours after cytokine injection. Expression was scattered diffusely throughout gray and white matter in cells which morphologically represent perivascular macrophages and/or vascular cells, glia, and small neurons. Immunohistochemical studies are in progress to identify these cells. There was no evidence of gliosis or infiltration by macrophages.

We conclude that endogenous cells in the CNS can be induced *in vivo* to express NOS II and that its expression in the spinal cord may play a role in transient thermal hyperalgesia. Supported by NS29226.

814.15

INTERLEUKIN-1ß INHIBITS *IN VIVO* AND *IN VITRO* ß-ENDORPHIN SECRETION FROM HYPOTHALAMIC NEURONS BY ACTIVATING NITROUS OXIDE SYNTHASE. N. Boyadjieva, A. Dey, S.J. Fung* and D.K. Sarkar. Dept. of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164-6520.

Interleukin-1ß (IL-1ß) is localized in the hypothalamus and responds positively after endotoxine administration. IL-1ß positive neuronal terminal fields included those in the arcuate nucleus. Since the arcuate nucleus contains a large population of ß-endorphin (ß-EP) neurons, we determined the effect of IL-1ß on *in vitro* and *in vivo* release of ß-EP from these neurons. Furthermore, the role of nitrous oxide synthase in the IL-1ß-regulated ß-EP secretion is determined, because nitrous oxide, which is produced by this enzyme, has been shown to mediate IL-1ß actions on various hypothalamic neuropeptides. *In vitro* release of ß-EP was studied using primary cultures of rat fetal mediobasal hypothalamic cells. IL-1ß concentration-dependently decreased the release of ß-EP from the cultured neurons. The inhibitory effect of IL-1ß was completely prevented by a nitrous oxide synthase blocker L-NAME. The blocker alone caused an increase in basal release of ß-EP from the cultured hypothalamic neurons. In agreement with these findings, IL-1ß and L-NAME significantly altered hypothalamic ß-EP release into the blood of pituitary portal vessels in ovariectomized rats. IL-1ß inhibited while L-NAME increased ß-EP levels in the blood of pituitary portal vessels. L-NAME also prevented the inhibitory effect of IL-1ß on pituitary portal blood levels of ß-EP. These data suggest that IL-1ß and nitrous oxide may interact to regulate ß-EP secretion from the hypothalamus. Supported by the National Institutes of Health Grant AA08757.

814.16

POTENTIATION OF CYTOKINE-INDUCED NITRIC OXIDE SYNTHASE ACTIVITY BY cAMP IN D30 MURINE ASTROCYTES. K.L. Burgher*, J. A. Heroux, and G. E. Ringheim, Neuroscience Product Group Unit, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

The effects of cyclic nucleotides on the expression of the inducible nitric oxide synthase (i-NOS) were investigated using an immortal astrocyte cell line (D30) derived from the mouse cerebellum. The expression of i-NOS activity from these cells was dependent upon co-stimulation with interleukin-1β (IL-1β) and interferon-γ (IFN-γ), and was not induced by either cytokine alone. The induction of i-NOS mRNA over basal levels was not apparent until 24 h and remained elevated out to at least 72 h. Accumulation of nitrite in the media from stimulated i-NOS activity was significant versus control cells by 48 h and continued to increase out to the latest time tested (72 h). Co-stimulation with 8-bromo-cyclic GMP (100 nM-100μM) alone or in combination with IL-1β and/or IFN-γ did not have any affect on the levels of i-NOS activity. Similarly, the addition of 8-bromo cyclic AMP (8-Br-cAMP), alone or in the presence of IL-1β or IFN-γ, did not result in detectable increases of i-NOS activity. In the presence of both IL-1β and IFN-γ, however, 8-Br-cAMP increased both enzyme activity and mRNA levels. The potentiation of i-NOS activity observed from co-stimulation with IL-1β and IFN-γ, either in the absence or presence of 8-Br-cAMP, was inhibited by preincubating the cells with L-N^5-(1-iminoethyl)ornithine, an irreversible i-NOS inhibitor. These results indicate that cAMP-mediated processes can synergize with cytokines to stimulate i-NOS expression in astrocytes. Moreover, since the requirement for either IL-1β and IFN-γ was not replaced by 8-Br-cAMP, additional signal transduction pathways are likely involved in the IL-1β and IFN-γ induced i-NOS expression.

814.17

IMPROVED PROCEDURE FOR IDENTIFYING NERVE FIBERS AND INDUCIBLE NITRIC OXIDE SYNTHASE IN RAT SPLEEN. J.C. Meltzer*, P.C. Grimm, A.Jansen, A.H. Greenberg, and D.M. Nance. Depts. of Anatomy, Pathology, Pediatrics, and Manitoba Inst. of Cell Biol., Univ. of Manitoba, Winnipeg, MB R3E 0W3.

Neuroimmune studies have demonstrated a critical role for the sympathetic innervation of the spleen as well as norepinephrine (NE) and neuropeptide Y (NPY) in modulating splenic immune function. As part of our analysis of neural regulation of splenic macrophage function, we have found that endogenous peroxidase activity (EPA) often precluded application of PAP immunohistochemistry to immune organs. We report procedures to reduce EPA and offer alternatives based upon alkaline phosphatase (AP) or Cy3 immunofluorescent detection systems. Rats were given splenic nerve cuts or sham surgeries and injected i.v. with saline or endotoxin and sacrificed at various intervals. Sections were digested with proteinase K (PK), placed in antibodies to the NE-synthesizing enzymes DBH and TH, NPY or inducible nitric oxide synthase (iNOS), and then developed with the various detection systems. Results indicated that protein digestion with PK increased the intensity and quantitiy of immunostaining and splenic nerve cuts eliminated TH, DBH and NPY fibers. Sensitivity with PAP, AP and Cy3 were similar for fiber staining, but EPA impeded interpretation of cellular iNOS staining induced by endotoxin. Thus, PK can be a valuable tool for antigen unmasking and AP and Cy3 offer an alternative detection system for organs with high levels of EPA such as the spleen. Supported by the MRC of Canada and the NIMH.

815.1

STATIC FORCE REPRESENTATION BY THE POPULATIONS OF MOTOR CORTICAL NEURONS IN THE JOINT-RELATED COORDINATE SYSTEMS.

S. Tanaka*. Dept. of Electrical Eng., Sophia Univ., Tokyo 102, Japan

Dynamics representation in the motor cortex has long been suggested. Experiments by Kalaska et al. (J Neurosci 1989) provided deeper insights into the relations of the activities of motor cortical neurons to dynamics parameters. The objective of the present research is to investigate theoretically static force representation in the motor cortex. In order to do it, the descending circuit from the motor cortex to the spinal cord is modelled, which relates the population activity of M1 pyramidal cells to that of motor neurons. The activities of the cortical neurons are modulated sinusoidally by the direction of static force represented in the intrapersonal, joint-related coordinate system (Tanaka, NSL 1994). The major consequences of the present theory are: (a) The populations of M1 cells code static force of the arm; (b) The direction of the force is coded distributedly throughout the whole populations; (c) The magnitude of the force is regulated by changing the depth of modulation of the activities of the M1 pyramidal cells; (d) The descending circuit transforms the static force coded in the motor cortex into static joint torques, which are coded by paired motoneuron pools in the spinal cord; (e) Change of the static joint torques with the postural change of the arm is provided by changing the activities of the pyramidal cells, rather than by changing the synaptic strengths of the neuronal circuit; (f) A substantial proportion (but not more than half) of the pyramidal cells has negative correlation with the static force, which was observed in experiments (for example Maier et al., J Neurophysiol 1993).

815.2

DISCRIMINATION AND CORRELATION OF EEG BRAIN ACTIVITY WITH STATIC TORQUE EXERTIONS IN THE UPPER EXTREMITY OF HUMAN SUBJECTS. J.P.A. Dewald*, A.L. Ricamato and J.D. Given. SMPP, Rehab. Inst. of Chicago, and Dept. of PM&R, Northwestern University Medical School, Chicago, Il.

We have combined EEG and EMG recordings during mechanically controlled static torque exertions in specific directions for the investigation of muscle synergies in the upper extremity of three subjects. Analysis of resulting spatial EMG profiles have shown that consistent muscle coactivation patterns exist across subjects. This work investigates the existence of similar distinct spatial organization at the level of the brain.

Subjects were seated with their wrist casted to a 6 degrees of freedom (D.O.F) load cell which measured the respective elbow and shoulder torques. Subjects were trained to produce elbow and shoulder torque ramps of 2 seconds duration followed by a 1.5 sec steady state hold using visual torque feedback. Four target directions, spaced apart by 90° and corresponding to elbow flexion-extension and shoulder abduction-adduction torques, were used in our paradigm. EMGs were recorded from 2 elbow and 6 shoulder muscles using surface electrodes. EEG signals were recorded from a total of 120 electrodes placed in a cap on the subject's head. The head and EEG electrode placements were digitized to permit additional EEG analysis. 80-100 trials of recorded EEG epochs were first aligned using either EMG or torque onsets and then ensemble averaged for each direction yielding SNR's > 15.

Alignment of EEG epochs using EMG onsets produced erroneous shifts of up to 200 ms between peak EEG motor potentials. These shifts disappeared when EEG ensemble averaging was realized using torque onsets. A comparison of subject specific 3D voltage maps, constructed from ensemble averaged EEG data and recorded head and EEG electrode 3D coordinates, showed differences in topographic brain activity as a function of target direction. However, for the same subject, topographical brain activity was consistent when repeating the same target direction.

These results indicate that ensemble averaging of EEG motor potentials is best achieved using torque onsets since torque provides the best measure of total motor output of the shoulder-elbow system. Furthermore, 3D voltage maps do indicate differences in brain mapping as a function of torque direction. This may indicate differences in topographical organization at the level of the brain prior to and at the start of the execution of different muscle synergies in the upper extremity. Supported by NIH NS19331.

815.3

CELL ACTIVITY IN MONKEY DORSAL PREMOTOR (PMd) AND PARIETAL AREA 5 CORTEX ARE ALTERED BY CHANGES IN ARM POSTURE FOR MOVEMENTS WITH SIMILAR HAND TRAJECTORIES. S.H. Scott, L.E. Sergio* & J.F. Kalaska. CRSN, Dépt. de Physiologie, Univ. de Montréal, Montréal, PQ, CANADA H3C 3J7

We have shown previously that neuronal activity in monkey primary motor cortex (M1) is altered by changes in arm posture for movements with similar hand trajectories (Scott & Kalaska, Neurosci. Abst. 20:982, 1994). The present study tested the effect of arm posture on cell activity in parietal area 5 (A5) and dorsal premotor (PMd) cortex. We trained two monkeys to move a pendulum-like handle to visual targets using two different arm postures- the 'natural' posture in the sagittal plane, and abducted approximately 80° into the horizontal plane in order to grasp and move the handle. The two arm postures changed the mechanical state (i.e. length and EMG activity) of muscles that span the shoulder and elbow joints. In both postures, the hand position remained at shoulder height. In both cortical areas, the activity of individual cells was usually not identical in the two postures. Tonic cell activity often significantly increased or decreased (PMd: 100/169 cells; A5: 45/80; M1: 390/539; F-test, $p<0.05$). The direction tuning of cells also often changed (PMd: 117/169; A5: 65/80; M1: 458/539; F-test, $p<0.05$) and the mean change in direction was less in PMd (22.6°) than in A5 (42.1°) or M1 (42.5°). Overall, the effect of arm posture was less for cells in PMd than in A5, and both these areas were less affected when compared to M1 or to mathematical models based on intrinsic movement attributes. Supported by MRC Group Grant in Neurological Sciences (JFK), and MRC (SHS) and FCAR (LES) Post-Doctoral Fellowships.

815.4

MAPPING MOTOR CORTEX OUTPUT ZONES WITH STIMULUS TRIGGERED AVERAGING OF EMG ACTIVITY: DISTAL AND PROXIMAL FORELIMB MUSCLE REPRESENTATIONS IN THE RHESUS MONKEY. JH Karrer*, BJ McKiernan, J Marcario, A Belhaj Saïf and PD Cheney Physiology Dept & Smith MRRC, Univ Kansas Medical Center, Kansas City, KS 66160

Using repetitive ICMS (intracranial microstimulation with high frequency trains) to evoke movements or EMG activity, others have reported significant areas of overlap in MI output zones representing distal and proximal muscles (e.g., Donoghue et al., Exp. Brain. Res. 89: 1, 1992). Such zones of overlap may serve as substrates for activation of functional muscle synergies underlying coordinated multi-joint movements. The goal of this study was to apply a more sensitive output mapping method – stimulus triggered averaging of EMG activity – to identity MI zones representing distal and proximal forelimb muscles. Two rhesus macaques were trained to perform two motor tasks: 1) a reaching and prehension task, and 2) a targeted push pull task. Averages of rectified EMG activity were computed from individual microstimuli (10-20 μA, 10-20 Hz) delivered to MI cortex in penetrations placed at 0.5 mm spacing. EMG activity was recorded from a total of 22 forelimb muscles including 11 proximal muscles (5 shoulder, 6 elbow) and 11 distal muscles (4 wrist, 5 forearm digit and 2 intrinsic hand muscles). Of the 728 stimulation sites tested, 464 produced effects in one or more of the target muscles. Of these sites, 157 produced effects exclusively in proximal muscles, 193 exclusively in distal muscles and 114 in both distal and proximal muscles. Consistent features were observed in MI output maps confirming work of others (Strick and Preston J. Neurophysiol. 48: 139,1982; Kwan et al., J.Neurophysiol. 41: 1120,1987). A primary distal output zone formed a central core that was surrounded medially, rostrally, and laterally by a horseshoe shaped proximal muscle representation. The medial arm of the proximal muscle representation showed overlap with the primary distal muscle representation. We conclude that stimulus triggered averaging of EMG is a sensitive method that can be used to map both excitatory and inhibitory zones in MI. Supported by NINDS grant #552096.

815.5

INPUT-OUTPUT PROPERTIES AND CHANGES OF RECRUITMENT GAIN OF THE CORTICOSPINAL PATHWAY. H. Devanne, B. A. Lavoie and C. Capaday*. Centre de Recherche en Neurobiologie, Université Laval, Québec, (Qc), Canada, G1J 1Z4.

Experiments were done to determine the form of the input-output relation of the corticospinal pathway to the motoneuron pools of the first dorsal interosseus (FDI) and the tibialis anterior (TA), respectively. The motor cortex was excited by focal transcranial magnetic stimuli (TCM). In both muscles the form of the input-output relation (i.e. stimulus intensity vs. response amplitude) was sigmoidal. The steepness of the relation increased whereas the threshold decreased with increasing tonic background activation up to about 30-40% of the maximum tonic effort. The plateau level was not influenced by the background activation level, except that it could not be reached when no background activity was present. This was probably due to the limitation of the stimulator maximum output. However, this finding may reflect a change in the intrinsic excitability of the motor cortex in going from rest to activity, or that convergent inputs from different descending systems are required for maximal activation of motoneuron pools. The sigmoidal input-output relation implies that the amount of facilitation or inhibition of a test EMR is a function of the test EMR amplitude and that in fact there is no range over which the amount of facilitation or inhibition is either constant, or relatively so, as has been suggested for monosynaptic spinal reflexes (see Crone et al., Exp. Brain Res., 81:35-45, 1990). The increase of the steepness of the relation with the background level of activity demonstrates that the recruitment gain (Kernell and Hultborn, Brain Res., 507:176-179, 1990) of the motoneuron pool changes as a function of the recruitment level even for the same qualitative task. This implies that adjustment of the stimulus intensity to compensate for the effect of changes of the background activity on EMR amplitude is not a valid procedure to insure that the amount of facilitation or inhibition be independent of the test EMR amplitude.

815.6

MODULATION OF NEURONAL ACTIVITY IN THE PRIMARY MOTOR CORTEX DURING PASSIVE TEXTURE DISCRIMINATION IN THE AWAKE MONKEY. **W. Jiang* and C. E. Chapman. CRSN, Université de Montréal, Canada.**

Previous studies have shown that area 4 neurones can encode surface texture and it was suggested that such sensory information is important in the control of precision grip under different conditions. The present study investigated the sensory properties of area 4 neurones while a monkey discriminated a change in the texture of surfaces passively applied to the contralateral fingertips. The monkey was trained to discriminate a standard surface (raised dots, 2 mm spatial period (SP) over the entire length) from 3 other surfaces in which the SP was proportionally increased to 3, 4 or 5 mm over the second half (surfaces physically continuous). Following the presentation of each surface, the monkey indicated the presence or absence of the change in texture by, respectively, pulling or pushing a lever with the opposite hand to obtain a juice reward. 41 out of 53 neurones tested either had a receptive field (RF) that included the scanning digit tips (14 touch, 8 pressure, 9 joint) or were vigorously active during discrete movements involving the scanning digits yet had no RF (n=10). Overall, 37 of the 41 units showed increased discharge to the presentation of the textured surfaces (4 cells not modulated) during the discrimination task. Texture change-related discharge was seen in only 3/37 neurones in the task, and all 3 had a cutaneous RF. 6 of the remaining 34 units were also tested for texture-sensitivity outside the context of the task (monkey not working; distracted with random drops of juice). 2/6 units (both had a cutaneous RF) now displayed texture-related discharge. The results indicate that texture-related information is indeed relayed to area 4, but texture sensitivity can vary as a function of the context within which the surfaces are presented. Supported by the MRC and Université de Montréal.

815.7

IMPORTANCE OF STIMULUS INTENSITY ON THE TIME COURSE AND MAGNITUDE OF MOVEMENT-RELATED SUPPRESSION OF TACTILE DETECTION IN HUMANS. Williams S.R., Shenasa J. and Chapman C.E*, CRSN, Université de Montréal, Montréal, Québec, Canada, H3C 3J7

Much debate surrounds the relative merits of active and passive touch. Previous studies have shown that active movement is accompanied by a reduction in the transmission of tactile cutaneous inputs. In order to evaluate the effect of a simple active movement on tactile detection of low intensity cutaneous stimuli, perceptual performance was examined in humans during the execution of a motor task, abduction of the right index finger. Stimuli were delivered to the glabrous tip of the right index finger. The intensity of stimulation was initially set at a level where 90% of the stimuli were stimuli were perceived at rest (P_{90}, 32 subjects). Four other stimulation intensities (1.25X P_{90} , 1.5X P_{90}, 1.75X P_{90} and 2X P_{90}) were examined in separate blocks of trials for 10 of the subjects. Data from individual subjects were pooled and the time course and amplitude of any movement-related suppression of tactile perception was examined in relation to two peripheral events: the onset of movement and the onset of movement-related electromyographic (EMG) activity. The time at which the first significant decrease in perception occurred increased from 210 ms before movement onset (190 ms before EMG onset) with stimuli of intensity P_{90} to 10 ms before movement onset (30 ms after EMG onset) at intensity 2x P_{90}. The minimum proportion of stimuli detected during movement trials varied between 0% (complete abolition of stimulus detection) at intensity P_{90} and 87% at intensity 2X P_{90}. These results indicate that movement-related suppression of tactile detection is highly dependant on the intensity of the stimulus being detected. Movement strategies in active touch could be designed to take advantage of this effect by optimising the signal to noise ratio in conditions where there is a widespread increase in absolute detection threshold.

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815.8

FUNCTIONAL CONNECTIVITY OF NEURONS IN THE SENSORY-MOTOR SYSTEM CHANGES DEPENDING ON THE PATTERNS OF VIBRISSAL MOVEMENTS IN RATS: ANALYSIS BY TIME DEPENDENT SCATTER DIAGRAMS. E. MIYASHITA[1]*, Y. HAMADA[2], M. OGURA[1] AND S. MORI[1]. [1]Lab. Neurobiol., Natl. Inst. Physiol. Sci., Okazaki, Aichi 444, Japan, [2]Lab. Neural Model., Fron. Res. Pro., RIKEN, Wako, Saitama 351-01, Japan

Functional connectivities among neurons in the rat vibrissal motor (MCx), sensory (SCx) cortices and the superior colliculus (SC) were studied in relation to the changes in patterns of vibrissal movements. In three rats, formvar-coated Nichrome wire electrodes (øout: 25 µm) were chronically implanted into the MCx, SCx and SC under ketamine hydrochloride anesthesia. After recovery from the anesthesia, multi-unit activities were recorded through these electrodes during sniffing movements of the vibrissae and during passive movements of them. Single unit activities were classified using a cluster cutting analysis. Functional connectivities of these units were analyzed by time dependent scatter diagrams (ref.: Y. Hamada et. al. in Brain Res. 660 (1994) 209–215). During sniffing movements of the vibrissae, both the neurons in the MCx and SCx had excitatory functional connectivities with neurons in the SC and the neurons in the MCx had inhibitory functional connectivities with the ones in the SCx. During passive movements of the vibrissae, both the neurons in the MCx and SCx had inhibitory functional connectivities with neurons in the SC and the neurons in the SCx had inhibitory functional connectivities with the ones in the MCx. These results suggest that the functional connectivities change depending on the behavioral states of the animals and the pattern of the changes is unique to a given behavioral state.

815.9

NEURONAL ACTIVITY IN MOTOR CORTEX OF CATS DURING THE PERFORMANCE OF FORELIMB MOVEMENTS. J.M. Criado*, A. de la Fuente, M. Heredia, A.S. Riolobos and J. Yajeya. Departamento de Fisiología y Farmacología, Universidad de Salamanca, Spain.

Findings from studies using recording from single neurons in awake animals, have revealed some specific functions for motor cortex. Our aim is to study the participation of motor cortex in different aspects of a voluntary movement. With this purpose, unitary activity has been recorded in motor cortex of cats previously trained in an associative conditioning. The process of conditioning consists in the presentation of an auditory stimulus (free field) with a random variable range of intensity (40dB, 70dB, or 100dB SPL). Three seconds later, the animal has access to the food by opening automatically a trap door. To eat, the cat must perform an extension movement of the forelimb followed by a flexion to get the food. Following this experimental protocol we studied the cellular discharge before and after the onset of movement of forelimb. Our results show that from twenty-eight cells tested, fifteen responded when the cat has the possibility to get the food. Onset latencies were in a great majority of cells circa 250 ms. Thirteen cells of them, keep their increased simple spike discharge during 1-2 sc, peaking circa one second after the trap door was opened. Also we have two cells which responses appeared before the opening of the trap. The results suggest two kinds of populations cells in motor cortex, ones related with voluntary movements and other with aspects related with the anticipation actions. (Supported by DGICYT Research Project PB 91-0421).

815.10

RETRODICTING SENSORY STIMULI AND PREDICTING MOTOR BEHAVIOR FROM NEURAL ACTIVITY

G. Gaál*, L.M. Kay and W.J. Freeman, University of California, Berkeley

Estimation of Jacobian matrices in nonlinear systems can be used to model visuomotor tasks and study population coding. Based on multiunit recordings in a visuomotor task from primate motor cortex, we developed a model for the task of reaching and tracking in a plane and showed how the model relates to recent population coding algorithms (Gaál, Physica D, 1995). Adaptive feedback control of joint angles of a three-joint arm was used. Visual error was combined with the transpose of the Jacobian matrix of the direct kinematic equation to determine the control signal. At first we simulated small movements at reference locations to estimate the Jacobian matrix. Then we estimated the matrix for simulated data by "reading off" changes in joint angles and x-y coordinate values of the hand from continuous simulated arm movement. In the present study, we apply the method of estimating Jacobian matrices for nonlinear functions of neural activity. We use "training" data sets recorded in an operant conditioning task from behaving rats. The rats presssed bars on olfactory cue for water reward (Kay and Freeman, Soc. Neurosci. Abstr., 1995). We then use the estimated matrices to retrodict sensory stimuli and predict behavior from "test" data sets.

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815.11

SINGLE NEURON ACTIVITIES DURING A SENSORIMOTOR DECISION **J. Zhang*[1], A. Riehle[2], S. Kornblum[1] and J. Requin[2] Department of Psychology, University of Michigan, Ann Arbor[1] and Cognitive Neuroscience Lab, CNRS, Marseille, France[2]**

A monkey was trained to perform wrist extension/flexion movements to align a pointer with a visual target while single unit activities in primary motor cortex (MI) were being recorded. The stimuli consisted of colored LEDs presented either to the left or right of a central starting position. Depending on the color of the LED, the monkey had to point either directly at it (compatible condition), or at LED on the opposite side (incompatible condition). Compatible and incompatible trials were blocked during training sessions, and were randomized within blocks during recording sessions. Neuronal activities in the four types of correct trials (left/right x compatible/incompatible) was analysed to determine whether such activity was more related to stimulus side, response side, or the compatibility rule that maps one onto the other. A 3-D vector was constructed to orthogonally decompose the *pattern* of neuronal activity (across four trial types) into the sensory, motor, and rule aspects of the task. The direction of the vector is represented by a point on the unit sphere that describes the functional loci of the activity (whether related to stimulus, to response, to rule, or to the monkey's "decision"), while the length of the vector reflects the differential activity (i.e., variance) across trial types and therefore its task-relevance. For each neuron, the peak differential activity during a trial was identified along with its spherical (functional) locus. We find that the peak spherical loci of the recorded population (156 units) in MI are clustered and evolve from sensory -> "decision" -> motor "landmarks" as a trial progresses, reflecting the monkey's sensorimotor transition during a trial of the task.

815.12

CONDITIONING OF MONKEY MOTORCORTICAL UNIT BIDIRECTIONALITY BY COMPLEXITY OF TRAINING PROGRAM. SA Sahrmann*, MH Clare, TW Anderson, M Yamaguchi, EB Montgomery, Washington University School of Medicine, Physical Therapy Pgm, Neurology Dept, St. Louis, MO 63110.

Motor cortical neuron discharge patterns have been related to movement elements such as force and direction. The relationship of the training task to the discharge patterns has not been studied. In this study, units in animals performing a complex repertory of graded and reversal ankle forces were compared with units in animals performing a simpler set of fixed force and direction tasks. Four rhesus monkeys were trained to perform ankle isometric force tasks in response to signal lights indicating magnitude and direction of force. Two animals performed a complex set of 4 tasks involving large and small forces and reversals of force. Two monkeys performed a simple two task set of unidirectional large forces. Standard extracellular recording techniques were used to record from hindlimb motor cortical area. Related cells (667) had a significant increase in firing rate ($p<.01$) timed with the large force phase, from rest to dorsal (D) or to plantar (P). Chi square was used to assess significant increase in unit firing rate and the distribution of cell types classified as unidirectional (UNI), D or P, or bidirectional (BI) both. Monkeys trained with the complex schedule had a greater proportion of BI units than those with the simple repertory ($p<.001$). The uni/bi ratios for complex training were 148/107 (1.38) and for simple training 311/101 (3.07). The proportion of Bi units for the small force task (Uni, 82; Bi, 50; ratio = 1.64) was similar to that of the large force task in the complex trained monkeys.

816.1

EFFECT OF A VIRTUAL REALITY ILLUSION ON MOTOR CORTICAL POPULATION VECTORS DURING DRAWING. D.W. Moran, A. Kakavand and A.B. Schwartz*. Barrow Neurological Institute, 350 W. Thomas, Phoenix, Arizona 85013; The Neurosciences Institute, 10640 John J. Hopkins Drive, San Diego, California 92121.

A motor illusion paradigm in a virtual reality environment was used to ascertain the relationship between visual, movement, and cortical population trajectories. A rhesus monkey was outfitted with stereographic goggles and trained to draw computer generated objects in free space. The 3D position of the monkey's hand was tracked in real time and downloaded to a graphics workstation that generated a virtual representation of the monkey's hand (spherical cursor) as well as the desired object to be drawn. The drawing task required the monkey to trace five continuous revolutions of a computer-generated ellipse. During the first two revolutions, the ratio of movement gains in the medial/lateral and superior/inferior directions was unity. During the third revolution, the movement gain in the medial/lateral direction was quasistatically increased such that a 1.0 cm hand movement caused a visual displacement of 1.8 cm. Given that the ellipse had a major/minor axis ratio of 1.8, the monkey visually perceived an elliptical trajectory during the remaining revolutions while, kinematically, its hand was moving in a circle. During repeated drawing tasks, single-cell recordings were obtained from the contralateral primary motor area. A population vector trajectory was constructed and compared to both the visual and movement trajectories.

Preliminary results from the analysis of 130 primary motor cortical cells in one hemisphere showed that the population trajectory was elliptical during the unity gain portion of the task and circular when the gain changed. Therefore, the population trajectory always matched the hand's trajectory. This suggests that motor cortical activity encodes the actual hand trajectory instead of the perceived movement. Supported by NIH (NS 26375).

816.2

CODING OF TARGET MOTION AND HAND MOVEMENT PARAMETERS IN MOTOR CORTEX DURING TARGET INTERCEPTION. W. Kruse*, N. Lindman Port, A.P. Georgopoulos. Brain Sciences Center, VAMC, and Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55417

We recorded the activity of 380 cells in the arm area of the motor cortex while a monkey intercepted a moving target on a computer screen using a 2D articulated manipulandum. The target accelerated, decelerated or traveled at a constant velocity for 0.5, 1 or 1.5 s. A multiple linear regression was used to relate the ongoing cell activity to the evolving position, velocity and acceleration of the target and hand movement. Since the interception movement was always upwards, the trial-by-trial time course of cell discharge at time t, was expressed as a function of the y-component of position, velocity and acceleration of the target, at time $t+k$, and the hand, at time $t+l$, where k and l were independent time shifts (-120 to $+140$ ms). The R^2 was calculated for each 10 ms shift. We found that in 378/380 cells the regression model was statistical significant at the combination of shifts with the highest R^2 (median $R^2 = 0.149$). In practically all cells both target and hand effects were significant; in only 1/378 cells there was only a significant hand but not target effect, and in only 2/378 cells there was a significant target but no hand effect. These results indicate that the motor cortex processes dynamically time-varying information concerning both target and hand movement. The same analysis was performed between the average (across cells) target and hand movement parameters and the y-component of the time-varying neuronal population vector. The highest R^2 (0.951) was obtained at $k = -90$ ms and $l = +100$ ms. The regression coefficients for position and velocity of target and hand movement were statistically significant ($p<10^{-4}$) but not those for target or hand acceleration. These results indicate that the moving target exerts an ongoing, dynamic influence on population activity with a latency of 90 ms, and that, in turn, the neuronal population exerts an influence on the hand movement with a latency of 100 ms.

816.3

CODING OF MOVEMENT PARAMETERS IN MOTOR CORTEX AND AREA 5: A COMPARISON USING TIME SERIES, MULTIPLE REGRESSION ANALYSIS. A.P. Georgopoulos*, J. Ashe. Brain Sciences Center, VAMC, and Departments of Physiology and Neurology, University of Minnesota Medical School, Minneapolis, MN 55417

A multiple linear regression model has been used to relate the ongoing cell activity in motor cortex and parietal area 5 to evolving parameters of movement (position, velocity, acceleration, and target direction) during 2D arm movements (Ashe, J. and Georgopoulos, A.P., Cerebral Cortex 6: 590, 1994). In the model the trial-by-trial time course of cell discharge at time $t+\tau$, was expressed as a function of the XY components of the target direction (unit length vector), and position (m), velocity ($m.s^{-1}$) and acceleration ($m.s^{-2}$) of the hand at time t, where τ was a time shift (-200 to +200 ms). The R^2 was calculated for each 10 ms shift. The regression coefficients of the XY components of each parameter can be used to construct a resultant vector the length of which describes the magnitude of the relation between the cell activity ($imp.s^{-1}$) and the particular movement parameter for each cell. Here we present the results of this analysis for 290 cells in the motor cortex and 207 in area 5 at the time shift for which R^2 was highest. In motor cortex and area 5, respectively, the magnitude (mean$\pm$SEM) of these vectors for the different parameters were as follows: position (124.4 ± 5.91 vs. 112.7 ± 6.70), velocity (31.2 ± 1.82 vs. 29.4 ± 2.07), acceleration (3.6 ± 0.24 vs. 3.3 ± 0.23), and target direction (15.0 ± 0.75 vs. 12.6 ± 0.76). Of these comparisons only the last one (target direction) was statistically significant ($P<0.05$, t test). These results indicate that the processing of time-varying movement parameter information is very similar in the motor cortex and area 5, except for target direction which exerts a stronger effect on motor cortical than on area 5 cell activity. (Supported by VA and NIH grants).

816.4

SET-RELATED ACTIVITY IN THE DORSAL PREMOTOR AREA REFLECTS TARGET LOCATION RATHER THAN LIMB TRAJECTORY. L. Shen* and G.E. Alexander. Dept. of Neurology, Emory Univ. Sch. Med., Atlanta, GA 30322.

The dorsal premotor area has been implicated in the preparation for movement, but the precise contribution of this region to motor control processes remains unclear. Single neuron activity was sampled from the dorsal premotor area and from primary motor cortex in two macaque monkeys while the subjects performed a visually-instructed, delayed reaching task. Subjects used the right forelimb to move a joystick, whose position was reflected by a cursor presented on a video display in front of the animal. Direct vision of the limb was prevented by an occluding collar. Each trial began with the illumination of a fixation point in the center of the display. After the subject had aligned the cursor with the fixation point, four radially arranged peripheral targets were also illuminated. After a variable delay, the subject was instructed, by the brief dimming of the appropriate peripheral target, which of the four would be the "correct" target for that trial. A second variable delay then ensued until the fixation point dimmed, which served as the movement- triggering stimulus. At this point, the subject was required to align the cursor with the correct peripheral target by moving the joystick in the appropriate direction. The direction of the target (relative to the fixation point) was dissociated from that of the hand/limb trajectory by varying the spatial mapping between joystick and cursor. This made it possible to determine whether the set- related activity that preceded the movement to capture the peripheral target was preferentially related to the direction of the forthcoming hand/limb movement or to the spatial properties of the target. Of the dorsal premotor area neurons with directional, set-related activity, nearly all showed directional tuning that reflected the direction/location of the target rather than that of the hand's trajectory. In contrast, in primary motor cortex roughly one half of the neurons with directional, set-related activity showed directional tuning that reflected the hand's trajectory while the other half reflected the direction/location of the target.

816.5

PHASIC RESPONSES TO SENSORY CUES IN THE DORSAL PREMOTOR AREA REFLECT ABSTRACT FEATURES OF THE INSTRUCTED MOVEMENT RATHER THAN SPECIFIC DETAILS SUCH AS MOVEMENT TRAJECTORY. G.E. Alexander* and L. Shen. Dept. Neurology, Emory Univ., Atlanta, GA 30322.

Previous studies have shown that neurons in the dorsal premotor area may respond to visual cues that serve as instructions or as triggers for the guidance of limb movements. There is general agreement that these responses are strongly conditioned by the motor instructional content of the stimulus, so that a premotor neuron may respond differentially to the same physical stimulus when it serves as the instruction for two different motor acts. Here we present evidence that this conditioning effect is based on a relatively abstract representation of the movement, namely the goal of the movement, rather than specific details such as the intended limb trajectory. Single neuron activity was sampled from the dorsal premotor area in two macaque monkeys while the subjects performed a visually-instructed, delayed reaching task. On each trial, the subject moved a joystick to align a cursor with a central fixation point, at which time four peripheral targets were also illuminated. After a variable delay, one of the four peripheral targets dimmed briefly. This served as the subject's spatial cue, instructing which target would be the "correct" one for that trial. A second variable delay then ensued until the fixation point dimmed, which served as the movement-triggering stimulus. The subject then aligned the cursor with the correct peripheral target by moving the joystick in the appropriate direction. To dissociate the direction of the target from that of the limb's trajectory, we varied the spatial mapping between joystick and cursor. Phasic neuronal responses to the spatial cue, when directional, were always tuned to the direction of the target, rather than that of the instructed limb movement. Phasic responses to the trigger stimulus were often directional as well, even though this stimulus contained no directional information. Like the responses to the spatial cue, directional responses to the trigger stimulus were always tuned to the direction of the target and not to the direction of the limb movement itself.

816.6

REPRESENTING SPATIAL INFORMATION FOR LIMB MOVEMENT: ROLE OF AREA 5 IN THE MONKEY. E. Guigon, F. Lacquaniti, L. Bianchi, S. Ferraina, R. Caminiti* INSERM CREARE, UPMC, 75005 Paris, France; Istituto Scientifico S. Lucia, 00179 Rome, Italy; Istituto di Fisiologia Umana, Universita La Sapienza, 00185, Rome, Italy.

How is spatial information for limb movement encoded in the brain? Computational and psychophysical studies suggest that hand position at start, via-points and targets is specified relative to the body to afford a comparison between the sensory (e.g. kinesthetic) reafferences and the commands that generate limb movement. Here we propose that the superior parietal lobule (Brodmann area 5) might represent a substrat for a body-centered positional code. Monkeys made arm movements in different parts of three-dimensional space in a reaction-time task. We found that the activity of area 5 neurons can be related to either the starting point, the final point, or combinations of the two. Neural activity is monotonically tuned in a body-centered frame of reference, whose coordinates define the azimuth, elevation and distance of the hand. Each spatial coordinate tends to be encoded in a different subpopulation of neurons. This parcellation could be a neural correlate of the psychophysical observation that these spatial parameters are processed in parallel and largely independent of each other in man.

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816.7

COMPARISON OF THE NEURONAL ACTIVITY IN SMA AND IN THE VENTRAL CINGULATE CORTEX DURING PREHENSION IN THE MONKEY. G.Cadoret* and A.M.Smith, C.R.S.N, University of Montreal, Quebec, Canada.

A total of 92 neurons in the cingulate cortex, and 115 neurons in SMA were found to be active in association with grasping and lifting in 2 *M.fascicularis* monkeys. Neurons in SMA were located in the medial wall, just caudal to the genu of the arcuate sulcus; neurons in the cingulate cortex were clustered in the ventral fundus of the cingulate sulcus caudal to the hand representation of SMA, in area 23c. The number of cells with proprioceptive fields was considerably higher than that of cells receiving cutaneous afferents in both the cingulate cortex (29:8) and the SMA (27:3). Hand movements could be evoked by ICMS at 8 recording sites in the cingulate cortex and at 12 sites in SMA. In the 2 samples of cells, the majority of the neurons increased their firing rate between 100 and 600 msec. before the grip onset, with the same distribution of onset times. The proportion of phasic cells observed in the 2 regions (62% in SMA and 65% in the cingulate cortex) was higher than in the motor cortex (52%). The proportion of phasic-tonic cells was lower in the cingulate cortex than in SMA. Force-pulse perturbations applied to the object during the holding phase elicited excitatory responses in 19% of the SMA neurons and 32% of the cingulate neurons, at a mean latency of 49 msec. in both groups of cells. No evidence of preparatory responses to the perturbation was found in the cingulate cortex, and only 5 cells in SMA exhibited a preparatory response. These results indicate that these 2 medial areas share some features and are involved in the sensorimotor control of the hand. However both regions seem to be insensitive to the preparatory grip force increases in anticipation of the perturbation. Supported by MRC of Canada.

816.8

DIFFERENTIAL INVOLVEMENT OF M1 AND A PREMOTOR AREA IN A MOTOR TASK. N. Picard*[1] and P.L. Strick[1,2]. [1]VAMC and [2]Depts. of Neurosurgery and Physiology, SUNY-HSC, Syracuse, NY 13210.

We examined activation of the cortical motor areas in monkeys performing motor tasks using the 2-deoxyglucose (2DG) method. Two monkeys were trained to perform remembered sequences of reaching movements for a juice reward (REM task). An additional two monkeys were trained on a Control task which required them to sit in a primate chair and lick juice rewards delivered at time intervals comparable to that of the REM task. Using serial sections spaced 90-100 μm apart, we generated flattened maps of 2DG uptake in motor areas located on the lateral surface and the medial wall of the hemisphere.

In animals performing the Control task, activation within the motor areas was largely confined to regions of face representation in the supplementary motor area (SMA) and primary motor cortex (M1). Activation related to licking also was present in animals performing the REM task. In addition, these animals had separate sites of activation in motor areas on the medial wall and lateral surface. The most extensive and intense activation was found bilaterally on the medial wall in the caudal cingulate motor area on the dorsal bank of the cingulate sulcus (CMAd). A smaller focus of activation was present in the rostral cingulate motor area (CMAr). On the lateral surface, activation was present contralaterally in the arm representation of M1. However, this activation was largely restricted to the anterior bank of the central sulcus. Surprisingly, the activation in M1 was modest in extent and intensity compared to that in the CMAd. In fact, the activation in the arm area of M1 was less intense than the activation in the face area associated with licking. Thus, the REM task appears to differentially recruit M1 and a premotor area (CMAd). Supported by the VA Medical Research Service, USPHS 24238 (PLS), and Medical Research Council of Canada (NP).

816.9

DIRECTION OF SACCADES EVOKED BY INTRACORTICAL MICROSTIMULATION (ICMS) OF SUPPLEMENTARY EYE FIELD (SEF) IS DEPENDENT ON BEHAVIORAL TASK CONDITION. N. Fujii [1,2], H. Mushiake [1], M. Tamai [2], J. Tanji*[1]. Department of Physiology [1], Department of Ophthalmology [2], Tohoku University School of Medicine, Sendai, 980, Japan.

In previous reports, the direction of saccades evoked by ICMS of the SEF has been characterized as goal directed, but at times as fixed vector. We report here that properties of saccades differ greatly depending on what oculomotor task the animal performs. A monkey (Macaca fuscata), facing a panel with five LEDs, performed a visually triggered saccade task with delay. When the animal placed its hand on a hold plate, an LED was turned on as a fixation target. If fixation was maintained for 1200-1600 ms (fixation period), the fixation LED was dimmed as a GO signal. An LED, serving as a saccade target, appeared about 500 ms before the GO signal, hence there was a delay between the appearance of saccade target and the GO signal. ICMS was delivered to the SEF at two different phases of the behavioral task. 1) During fixation, 500 ms after the initiation of the fixation. 2) immediately (50-100 ms) before the GO signal (pre-GO signal).

During fixation, the direction of stimulus evoked saccades was always contralateral to cortical stimulus sites, e.g., from the left SEF, the evoked saccades were directed rightward. The saccade trajectory appeared either goal directed or constant vector, with a mean latency of 124.7 ms. In contrast, when the pre-GO ICMS was applied, the direction of evoked saccades altered depending on the position of the precued target. Thus, the animal captured the target with a saccade, despite the presence of the ICMS. If the saccade target was presented contralateral to the stimulus site, the interval between the GO signal and the saccade onset was <100 ms. If the target appeared ipsilaterally, the interval ranged from 150-300 ms.

816.10

A MODEL FOR SHIFTING RECEPTIVE FIELDS BASED ON GAIN-MODULATION AND HEBBIAN LEARNING. E. Salinas, and L.F. Abbott*. Center for Complex Systems, Brandeis University, Waltham, MA 02254.

Multimodal cortical neurons implicated in a number of spatial tasks involving coordinate transformations exhibit two types of receptive fields: (1) gain-modulated and (2) shifting. Gain-modulated responses can be modeled as the product of a conventional receptive field and a variable gain factor (Andersen et al., Science, 1985). Neurons in parietal area 7a combine visual, eye-position and head-position information in this manner (Andersen et al.). In contrast, some neurons in premotor cortex responsive to somatosensory and visual stimuli have visual receptive fields that shift when the arm or head moves, but not when the eyes move (Graziano et al., Science, 1994). In area LIP, visual receptive fields that shift in anticipation of a saccade have been recorded (Duhamel et al., Science, 1992). Auditory receptive fields that shift with eye position have also been reported (Stricanne et al., Neurosci. Abstr., 1994).

We show, using theoretical methods and computer simulations, that neurons receiving inputs from gain-modulated cells can automatically develop shifting receptive fields if input synapses are subject to a Hebbian or correlation-based synaptic modification rule. No dynamic reconnection or other type of switching mechanism is necessary if the presynaptic neurons have gain-modulated responses. This is consistent with the fact that gain-modulated cells project extensively into areas that exhibit shifting receptive fields. The synaptic weights needed to produce shifting responses develop spontaneously without any supervision or error signal. The model makes testable predictions about how the responses of neurons with shifting receptive fields might be modified by training. In general, gain-modulated neurons provide a powerful coordinate-free representation from which downstream networks can extract the information relevant to their function by means of correlation-based synaptic modification. Supported by grant NSF-DMS9208206.

816.11

OSCILLATIONS IN MONKEY MOTOR CORTEX DURING VISUALLY- AND MEMORY-GUIDED REACHING MOVEMENTS. B. Acuna*, C. Ojakangas, J.N. Sanes and J.P. Donoghue. Department of Neuroscience, Brown University, Providence, RI 02912.

We previously described γ-range (25-50 Hz) local field potential oscillations LFPs) in monkey primary motor cortex (MI, Sanes and Donoghue, PNAS 90:4470, 1993). The γ-LFPs occurred throughout a pre-movement delay period, ceasing around movement onset. They also emerged when the monkey changed behavior from quiet sitting to task engagement, suggesting that they are related to motor preparatory states. The current experiment tested the hypothesis that MI γ-LFPs encode remembered movement direction. We recorded γ-LFPs in MI through chronically implanted microwires in a monkey performing visually guided reaching movements to 1 of 3 targets represented on a video monitor. At first, the monkey position the hand at a central zone for an initial hold period in each of two tasks. In the *memory task*, 1 of 3 visual targets was then illuminated for 800 ms. After a 4 s "pre-cue" period, all targets were displayed, and the monkey was required to move a position feedback cursor to the instructed target within 600 ms after the go-cue. In the *visual task*, the target remained illuminated throughout the pre-cue period. In both conditions, distinct γ-LFPs were evident during both the hold and pre-cue periods, but they diminished around movement onset and remained low throughout the movement. We conclude that γ-LFPs are not related to preparation for a specific movement direction because they occurred during the hold period (before movement direction was specified), and they showed no directional tuning. Additionally, no apparent difference in the pattern or occurrence of γ-LFPs was seen between the memory and visual tasks. Synchronously active γ-LFPs were sometimes evident across multiple sites, but these were not temporally locked to any task event. These findings indicate that oscillations are not related to details of upcoming learned motor actions or to memory processes that might occur within MI, but that they are inversely related to motor execution. Supported by Grant NS 25074.

816.12

DYNAMIC INTERACTIONS BETWEEN NEURONS OF THE MONKEY MOTOR CORTEX IN RELATION TO BEHAVIORAL EVENTS. A. Riehle[1]*, S. Grün[2], A. Aertsen[2], J. Requin[1]. [1]Cognitive Neuroscience Lab., CNRS, Marseille, France; [2]Dept. Neurobiol., Weizmann Inst. of Science, Revohot, Israel.

Simultaneous recording of adjacent motor cortical neurons reveals rapid modifications in their synchronized activity during the execution of various motor tasks. These modulations are time-locked to the occurrence of either external events, such as the presentation of visual stimuli, or internal events, such as the time when the stimulus is expected and/or the movement is executed. Coincident firing enhances the probability that the receiving neuron will be activated. Hence, coincident firing might be considered as a potential neural code used to bind otherwise disparate activity into a coherent entity. Dynamics of interactions between neurons were studied both by using trial-averaged measures (Joint PSTH [1]) and by analyzing, trial by trial, individual epochs of synchronized firing ("unitary events" [2]). "Unitary events" were defined as activity constellations that occur more often than expected by chance on the basis of the firing rates of the neurons. The presence and significance of these events were evaluated, taking also into consideration the non-stationarity in the firing rates of the neurons involved. The results suggest that the degree of synchronization in groups of motor cortex neurons might play a role in the neuronal computations underlying the execution of a motor task. Furthermore, the time structure of the interaction between neurons supports the hypothesis that a single neuron might participate in different neuronal groups by rapidly changing its coupling to other neurons as a function of computational demands.

[1] Aertsen A, Gerstein G, Habib M & Palm G. J. Neurophysiol. 61:900-917 (1989) ; [2] Grün S, Aertsen A, Abeles M, Gerstein G & Palm G. Europ. J. Neurosci., Suppl. 7:11 (1994)

816.13

PRECISE SPATIO-TEMPORAL FIRING PATTERNS IN THE FRONTAL CORTEX OF BEHAVING MONKEYS: EXISTENCE AND CORRELATION TO BEHAVIOR. Y. Prut, E.Vaadia*, H. Bergman, I. Haalman, H. Slovin, and M. Abeles. Dept. of Physiology and the Center of Neural Computation, The Hebrew university, Jerusalem 91010, Israel

The possibility that temporally precise events participates in cortical information processing was tested. Multiple single units were recorded in the frontal cortex of Rhesus monkeys during performance of a delayed response task. We searched for firing patterns (FPs), defined as repeated spike-triplets with specific time intervals (with jitter of ±1 ms). The estimation of significance took into account the firing rates and the pairwise correlation amongst the units.

We found that: **(1)** FPs were composed of spikes of one or more units, spanning few to hundreds ms. A given triplet of units exhibited FPs, of different temporal structure.**(2)** Many FPs appeared in relation to behavior. Moreover, different FPs of the same units could appear preferentially during different behavioral modes. **(3)** FPs tended to appear in clusters, each dominated by a different set of units. The clusters were also related to the behavior. **(4)** Cross-correlations (CC) were computed for spike trains and compared to the corresponding 'FPs triggered' CCs. For about 60% of the neuronal pairs, the two CCs had different shapes. **(5)** The range of observed time intervals in FPS of a given neuronal pair, was either broad (presumably corresponding to a weak coupling) or narrow (presumably corresponding to a strong coupling). **(6)** Some FPs appeared more frequently in association with the occurrence of certain other FPs.

The findings support the hypothesis that information processing is mediated by synchronous activations of neuronal groups, allowing single neuron to participate in different computational processes.

816.14

NEURAL NETWORK MODELING OF MOTOR CORTICAL OPERATIONS DURING MENTAL ROTATION AND MEMORY SCANNING TASKS. A.V.Lukashin*, B.R.Amirikian, V.L.Mozhaev and A.P.Georgopoulos. Brain Sciences Center, VA Medical Center, Minneapolis, MN 55417

We propose a neural network model that reproduces quantitatively the spiking activity of motor cortical cells recorded in behaving monkeys during performances of two different visuomotor tasks: (i) the mental rotation task, which required the production of a movement at an angle from a stimulus direction, and (ii) the memory scanning task, which required the selection of an appropriate movement direction, depending on the serial position of stimuli in a sequence. In the present study, the ensemble of directionally tuned motor cortical cells is simulated by a recurrent network of interconnected, stochastic spiking neurons. The key point of the model is that a large repertoire of neural activity is permanently stored in the connectivity matrix in such a way that, once it is initiated, a particular neural dynamics evolves in time as a self-sustained dynamic attractor of the network. Our simulations demonstrate that specific patterns of motor cortical activity observed in experiments using paradigms (i) and (ii) can be stored in the connectivity matrix and can be initiated by specific external inputs of short duration.

816.15

HIGHLY NONLINEAR PROCESSING OF TEMPORAL INPUT FLUCTUATIONS BY REALISTICALLY MODELED CORTICAL BURSTING NEURONS P.H. Bedenbaugh* and M.M. Merzenich, *Keck Center for Integrative Neuroscience, Univ. of California at San Francisco, San Francisco, CA*

Analytical models of spiking neurons, MacGregor (1987) model neurons, and sigmoid elements all misrepresent and nonlinearly distort temporally fluctuating inputs (Bedenbaugh, 1993). Inputs to these models must therefore be highly synchronous to produce synchronous outputs. All of these models have simple dynamics dominated by the kinetics of post-synaptic potentials. Through computer simulation, we studied whether or not the richer intrinsic properties and longer time constants (*eg.*, as provided by calcium exchange, diffusion and modulation) of a bursting neuron would represent temporally fluctuating inputs more accurately than do these simpler models.

A one-compartment model of a cortical bursting neuron based on a model of Lytton and Sejnowski (1991) was simulated with NEURON. Pairs of model neurons received input from partially overlapping pools of Poisson spike trains. Simulations were run with both low variance input (1000 presynaptic neurons) and high variance input (100 presynaptic neurons). For both sets of simulations, the synaptic strength was varied to evoke firing rates ranging from less than 4 spikes per second to over 90 spikes per second. We measured the output correlation coefficient as a function of the fractional shared input. These quantities would be equal for a system that represents temporally fluctuating inputs well.

In every case, the output correlation coefficient was far less than the fraction of shared input, just as for the more simplified models. We conclude that neurons that behave like this model neuron are better suited for responding unambiguously to particular synchronized spatial input patterns than to accurately representing fluctuating inputs with a fluctuating output spike train.

Special thanks to Michael Hines for help with NEURON. Thanks to Bill Lytton for the calcium pump/diffusion model. Supported by NIH DC00144 and Pittsburgh Supercomputing Center.

816.16

NON-LINEAR DYNAMICS OF SPONTANEOUS NEOCORTICAL FIELD POTENTIALS RECORDED DURING ANESTHETIZED AND AWAKE STATES IN CHRONICALLY IMPLANTED RATS. Mark E. Jackson* and Larry J. Cauller, GR41, Neuroscience Program, University of Texas at Dallas, Richardson, TX 75083-0688.

Nonlinear dynamics provides a means of analyzing multi-variable, complex systems that were formerly considered either random or too complex to understand. The analysis of the correlation dimension of a one-dimensional time series can reveal the deterministic nature of the complex system and allows an estimation of the minimum number of variables necessary to describe that system. We are using non-linear dynamics to analyze the cortical activity of rats with the goal of determining the essential neurophysiological constituents of functional cortical activity. This study developed an intact rat preparation with chronically implanted electrodes in SI, MI, and AI neocortex. Spontaneous field potentials were recorded during deep Nembutal anesthesia and during non-moving, alert activity. The autocorrelation function showed a slow fall-off and an extended range of positive correlation and the power spectrum showed broadly spaced peaks, typical of a fractal signal. Samples of the time series (32 sec at 1 msec sampling interval) were analyzed using the Grassberger-Procaccia method to determine the correlation dimension. The dimension in the awake state was higher than the anesthetized state (≈2.9 vs. 1.2). We plan to use this preparation to study cholinergic modulation in the neocortex of awake, behaving rats. Supported by a grant from the Whitehall Foundation.

BASAL GANGLIA: BEHAVIOR

817.1

BLOCKADE OF HALOPERIDOL-INDUCED CATALEPSY BY 8-OH-DPAT IS MEDIATED BY FOREBRAIN RECEPTORS. K. Eberle-Wang*, R.J.A. Banks and M-F. Chesselet. University of Pennsylvania, Department of Pharmacology, Philadelphia, PA 19104.

Peripheral administration of the prototypical dopamine receptor antagonist, haloperidol, elicits catalepsy in rats. Studies by Neal-Beliveau et al. (1993) demonstrated that systemic administration of the 5-HT_{1A} agonist, 8-OH-DPAT, blocked haloperidol-induced catalepsy. The location of the 5-HT1A receptors mediating this response is unknown. In the present study, we have compared the effects of 8-OH-DPAT administration into the lateral ventricles (to act on forebrain 5-HT_{1A} receptors) vs. infusion into the cisterna magna (to act on spinal chord 5-HT_{1A} receptors) on haloperidol-induced catalepsy. Male Sprague-Dawley rats were implanted with chronic guide cannulae for intracerebroventricular (ICV) or intracisternal (ICM) drug administration. All rats received 3 once-daily injections of haloperidol (2.0 mg/kg, s.c). Five days later, the animals received the same dose of haloperidol and catalepsy was recorded at 1, 2, 3 and 4 hours after injection. To test for catalepsy, rats were placed with forepaws resting on a horizontal bar 11 cm above the bench top and the latency to replace both forepaws on the bench top was measured (max 300 sec). Fifteen min prior to the 3 hour observation, 8-OH-DPAT (10 µg/5 µl) or saline was administered ICV or ICM according to a within-subjects design. ICV infusion of 8-OH-DPAT significantly decreased by 81% the duration of catalepsy at the 3 hour time point, 15 min after its infusion. However, catalepsy returned to control levels by the fourth observation period, 75 min after 8-OH-DPAT infusion. In contrast, ICM infusion of the same dose of 8-OH-DPAT had no effect of haloperidol catalepsy. These data confirm and extend previous studies demonstrating that 5-HT_{1A} agonists block haloperidol-induced catalepsy in the rat. Furthermore, the data suggest that 5-HT_{1A} receptor mechanisms in the forebrain, rather than in the spinal chord, are responsible for mediating the anticataleptic effect of 8-OH-DPAT. Supported by PHS grants MH44894 and MH48125 and Tourette Syndrome Association.

817.2

ΔFosB PARTICIPATES IN THE MEDIATION OF PRIMING G.S. Robertson* and M. Morelli[2] Dept. of Pharmacology[1], University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5. Dept. of Toxicology[2], University of Cagliari, Viale A Diaz 182, Caligari 09100, Italy.

Administration of dopamine receptor agonists to rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway produce changes in the denervated striatum that enable a subsequent injection to elicit more vigorous circling. This behavioural phenomenon is termed priming. D1-like dopamine receptor agonists profoundly elevate immediate-early gene (IEG) expression in the denervated striatum. IEGs encode transcriptional regulating factors suggesting they may participate in gene signalling pathways that mediate priming. Indeed, we have recently discovered that chronic alterations in dopaminergic neurotransmission persistently enhance expression of the IEG product ΔFosB in the striatum. In order to determine whether ΔFosB is involved in priming, we examined whether (A) levodopa-induced priming and ΔFosB expression have similar time courses and (B) if inhibition of ΔFosB synthesis with an antisense oligonucleotide reduces levodopa-induced priming. ΔFosB expression in the 6-OHDA-denervated striatum peaked 3 days after levodopa administration and returned to preinjection levels at 10 days. This time course is similar to that for levodopa-induced priming. Moreover, intrastriatal injection of an antisense, but not a random, oligonucleotide against Δ*fos*B mRNA 14 hours prior to levodopa administration significantly reduced circling to a subsequent injection of the selective D1-like agonist SKF 38393. Taken together, these findings suggest that ΔFosB may play a role in those intracellular events which mediate levodopa-induced priming.

817.3

THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS AS A STRIATAL OUTPUT STATION. I. EFFECTS OF MICROINJECTIONS OF GABA AGONISTS AND ANTAGONISTS ON STEREOTYPED BEHAVIOR. L.F. Allen,* M.P. Latimer, R.I.J. McConnell, A.S. Walls and P. Winn. School of Psychology, Univ. St Andrews, Fife, Scotland KY16 9JU.

Excitotoxic lesions of the PPTg disinhibit orofacial stereotypy stimulated by *d*-amphetamine in the ventrolateral caudate-putamen (VLCP) *(Allen LF and Winn P, Exp. Brain Res. 1995, in press)*. Anatomical and behavioral evidence indicates that outflow from both dorsal and ventral striatum is processed through the PPTg but it is not clear what particular role this structure fulfils. There are several VLCP outflow sites from which orofacial stereotypy can be stimulated by microinjecting GABAergic drugs. In the present experiments we have sought to first, confirm that microinjections of GABA drugs into the substantia nigra pars reticulata (SNr) elicit orofacial stereotypy; and second, examine the effects of various doses of the GABA agonist muscimol and the antagonist picrotoxin microinjected into the PPTg. In each case rats' performance was videotaped and scored using a checklist. In the first experiment, 75ng/0.5µl muscimol injected into anterior SNr stimulated licking and biting, directed mainly at the cage environment. Injections into posterior SNr had significantly less effect. 75ng/0.5µl picrotoxin had no effect on orofacial activity when injected into either anterior or posterior SNr. In the second experiment, 15, 30 and 45ng/0.3µl of either muscimol or picrotoxin microinjected into the compact portion of PPTg had no effect on orofacial stereotypy. Small changes in the incidence of sniffing and rearing were observed. These data suggest that while loss of the PPTg disinhibits orofacial stereotypy stimulated from the VLCP, manipulating GABA transmission in the PPTg in the absence of other stimulation does not induce significant changes in orofacial or other forms of unconditioned behavior.

817.4

THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS AS A STRIATAL OUTPUT STATION. II. EFFECTS OF PPTg LESIONS ON REACTION TIME MEASURES IN A CONDITIONED VISUAL DISCRIMINATION TASK. P. Winn,* M.P. Latimer, V.J. Brown and P.J. Brasted. School of Psychology, Univ. St Andrews, Fife, Scotland KY16 9JU.

Anatomical, functional imaging and behavioral data indicate that the PPTg is an important output station for the dorsal striatum and related structures. In the present experiments we examined the role of the PPTg in mediating reaction time (RT) responses in a conditioned visual discrimination task known to be sensitive to dorsal, but not ventral, striatal dysfunction. Rats were trained in a nine-hole box to make responses from a central start-point: bright stimulus lights signalled a right side response, dim lights a left side response. Correct responses were rewarded by 45mg food pellets. The time taken to leave the central start point after stimulus onset was recorded as RT. Before lesions were made there were no differences between rats in RT; bilateral ibotenate lesions of PPTg were then made (0.12M: 2 X 0.2µl injections/hemisphere; sham lesions 2 X 0.2µl PBS; unilateral lesions were made, followed 24h later by lesions on the contralateral side). After surgery significant differences were found. Lesioned rats were significantly slower in RT whereas control operated rats showed no change from preoperative performance. Histological analysis showed that both cholinergic (identified by NADPH diaphorase histochemistry) and non-cholinergic PPTg neurons had been damaged. These data (i) confirm that the PPTg is involved in the mediation of dorsal striatal processes; (ii) can be explained by suggesting either an interference with striatal outflow systems (principally directed through non-cholinergic PPTg neurons) or by suggesting that arousal and/or activational processes mediated by PPTg cholinergic neurones have been affected.

817.5

Behavioral Effect of Focal Inhibition of the Subthalamic Nucleus. D. Dybdal, K. Japikse, D. Burnhill, and K. Gale*. Dept. Pharmacology, Georgetown Univ. Med. Ctr., Washington DC, 20007.

Previous electrophysiological studies have suggested that the subthalamic nucleus (STN) is an important source of excitatory glutamatergic input into substantia nigra (SN). To evaluate the functional significance of these inputs in the awake, behaving rat, we compared the effects of focal pharmacological inhibition of STN with the effects of blocking glutamate receptors within SN. Infusion of muscimol (200pmol), a $GABA_A$ agonist, unilaterally into STN induced a pronounced contraversive postural asymmetry without stimulation of locomotor activity. A similar response was evoked by the direct infusion of kynurenate (100nmol), a glutamate antagonist, unilaterally into SN. These results are consistent with the hypothesis that excitatory glutamatergic inputs into SN regulate postural control and that the STN is a source of these inputs .

The absence of locomotor stimulation following the inhibition of STN or blockade of glutamate transmission in SN was in clear contrast to the marked locomotor stimulation (in addition to postural asymmetry) induced in the same animals following direct unilateral application of muscimol in SN, an effect that has been previously well characterized. It therefore appears that while STN-derived glutamate inputs regulate postural adjustments, non-glutamatergic excitatory influences derived from regions other than STN must contribute to the nigral regulation of locomotor activity.

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817.6

MODULATION OF METABOTROPIC GLUTAMATE AGONIST INDUCED ROTATION BY ADENOSINE AGONISTS AND ANTAGONISTS. J. Feeley Kearney* and R. L. Albin. Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, MI, 48109-1687.

Metabotropic glutamate receptors (mGluRs) are a major class of excitatory amino acid receptors. Eight mGluR subtypes coupled to a variety of effector systems have been cloned. Binding studies have shown that the striatum and subthalamic nucleus (STN) possess a high density of mGluR binding sites (Albin et al, 1992, Neurosci, 46, 35). Unilateral intrastriatal and intraSTN infusion of the mGluR agonist 1-aminocyclopentane-1S,3R-dicarboxlyic acid (ACPD) induces vigorous contralateral rotation in rats. There is a high density of adenosine A_2 receptors in the striatum (Jarvis et al, 1989, Eur J Pharmacol, 18, 243), and some of the effects of striatal mGluR activation may be mediated by modulation of adenosine effects. We performed a series of experiments to determine if the contralateral rotation induced by unilateral striatal mGluR activation can be modified by activation or blockade of adenosine receptors.

Pretreatment with the adenosine antagonist theophylline (25mg/kg) significantly decreases contralateral rotation induced by unilateral intrastriatal ACPD (1µmol). Intrastriatal and intraSTN coinjections of theophylline (5mM) and ACPD (1µmol) significantly decrease contralateral rotation suggesting that mGluRs and adenosine receptors interact locally. These appear to be adenosine A_2 effects as the selective A_2 antagonist 8-(3-chlorostyryl)caffeine (CSC) (3mg/kg) also decreases contralateral rotation induced by unilateral intrastriatal and intraSTN ACPD, while the selective A_1 antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (5mg/kg) has no effect. Pretreatment with the selective A_2 agonist CGS 21680 potentiates contralateral rotation induced by unilateral striatal ACPD, whereas pretreatment with the selective A_1 agonist N6-cyclopentyl-adenosine (CPA) has no effect. These results suggest that mGluR effects may be due, in part, to modulation of adenosine action.

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817.7

THE LOCATION OF AN IBOTENIC ACID LESION IN THE DORSAL STRIATUM AFFECTS THE BEHAVIOURAL DEFICIT OBSERVED. R.A. Fricker*, L.E. Annett, E.M. Torres and S.B. Dunnett MRC Cambridge Centre for Brain Repair, University of Cambridge, UK

The excitotoxic model of striatal damage has been used extensively in the rat to test motor deficits both in whole body asymmetries and in skilled limb movements. However, conflicting results of both the type and extent of behavioural deficits have been reported. Unilateral excitotoxic lesions of the dorsal striatum, typically give rise to ipsilateral rotation in response to both amphetamine and apomorphine. Conversely, recent studies have claimed that a lesion in the rostral striatum induces a marked contralateral rotation in response to apomorphine. Bilateral excitotoxic lesions placed in the dorsolateral striatum cause a marked impairment in reaching movements of the forelimbs and tongue and lesions to more ventrolateral regions result in impairments in the initiation and execution of these movements. The present study investigated the effect of unilateral ibotenic acid lesions in the dorsal striatum on rotation in response to both amphetamine and apomorphine and in the "staircase test" of skilled forelimb use. In a 2x2 matrix design experiment, adult female Sprague-Dawley rats received a double unilateral lesion of 0.5 µl 0.06M ibotenic acid at 2 sites in either the anterior, posterior, medial or lateral striatum. Rats which received posterior lesions showed a marked ipsilateral rotation in response to both amphetamine and apomorphine, while animals receiving anterior lesions showed little ipsilateral or a slight contralateral bias. Rats receiving lateral lesions showed a marked impairment of contralateral paw use on the "staircase test" while animals with medial lesions showed no significant difference to control unoperated animals. These results confirm the somatotopic organisation of the dorsal striatum in its role in motor functions and indicate the necessity for design of lesion and transplantation studies to take into account the locus of an excitotoxic lesion in order to evaluate the behavioural deficits and recovery which result.

817.8

PREFERENTIAL LOCALIZATION OF SELF-STIMULATION SITES IN STRIOSOMES/PATCHES OF RAT CAUDATE-PUTAMEN. N.M. White* and N. Hiroi, Department of Psychology, McGill University, Montreal, Quebec, Canada.

The striatum consists of two histochemically distinguishable compartments: "patches" or "striosomes", surrounded by a "matrix". As a test of the hypothesis that the striosome/patch compartment may have reward-related functions, this study examined the role of the two striatal compartments in electrical self-stimulation of the brain. Rats were implanted with monopolar electrodes aimed at the medial portion of the caudate-putamen. After recovery from surgery they were trained to bar press for 0.5 sec trains of 60Hz sine-wave stimulation. Training consisted of placing the rats in the test cage and "shaping" them to press the bar by administering trains of stimulation contingent upon behaviors that approximated the bar pressing response. Following each training session each rat remained in the cage for 30 min to test for spontaneous bar pressing. All rats were trained until they reached a self-stimulation criterion -- at least 100 spontaneous responses in 30 min on 3 consecutive days -- or for a maximum of 11 - 15 days. After attaining or failing to attain the criterion the animals' brains were stained for calbindin or calretinin. Animals with electrodes that were in or touching areas of light calbindin (Gerfen, et al, Proc.Natl.Acad.Sci.USA 82:8780-8784,1985) or heavy calretinin (Hiroi, in preparation) staining, indicating contact with a patch/striosome, acquired self-stimulation behavior more reliably than animals with electrodes terminating exclusively in tissue expressing heavy calbindin or light calretinin staining (matrix). The results provide *in vivo* evidence that the striosome/patch compartment is functionally differentiated from the matrix compartment. Direct stimulation of striosome/patch compartment, but not of the matrix, led to acquisition of bar pressing behavior, either because striosome stimulation has rewarding consequences, or because stimulation of this area promotes the storage of new information.

817.9

INTRASTRIATAL DNQX INDUCES CONTRALATERAL ROTATION AND GLOBUS PALLIDUS FOS IN DOPAMINE-DENERVATED RATS J.J. Schuller* and J.F. Marshall, Dept. of Psychobiology, University of California, Irvine, 92717.

The striatopallidal and striatonigral projections are the two major output pathways of the striatum. These pathways both use GABA as a neurotransmitter, and are influenced by glutamatergic and dopaminergic afferents. Several measures of neuronal activity indicate that interruption of dopaminergic neurotransmission in the striatum leads to increased basal activity in striatopallidal neurons. We have examined the role of striatal glutamate in mediating the striatopallidal hyperactivity by combining local glutamate antagonist application with behavioral quantification and immunocytochemical localization of Fos in the globus pallidus (GP) as an index of striatopallidal activity. Rats were given unilateral 6-OHDA lesions of the nigrostriatal pathway. 24 d post-lesion, each rat was implanted unilaterally with a guide cannula aimed at the anterior portion of the denervated striatum. Unlesioned control rats were also implanted with striatal cannulae at this time. 3-4 d following cannula implantation all rats were infused intracerebrally with the AMPA receptor antagonist DNQX (0.75, 1.5, or 3 ug) or vehicle (1ul), and perfused 2 h later. The fixed brains were sectioned and processed for Fos immunoreactivity. Rats with 6-OHDA lesions displayed dose-dependent contralateral rotation in response to DNQX, but not to its vehicle. Unlesioned control rats were unresponsive to either DNQX or its vehicle. DNQX dose-dependently induced Fos in the GP of 6-OHDA-lesioned rats but not in unlesioned control rats. Vehicle did not induce significant GP Fos in either lesioned or unlesioned rats. Based on these data we conclude that glutamate acting at striatal AMPA receptors contibutes to DA-denervation-induced hyperactivity in the striatopallidal pathway. Furthermore, the disinhibition of pallidal neurons may play an important role in the expression of motor behavior.

817.10

TIME- AND DOSE-DEPENDENT EFFECTS OF THIO END-CAPPED ANTISENSE OLIGONUCLEOTIDES ON AMPHETAMINE-INDUCED STRIATAL *c-FOS* EXPRESSION AND BEHAVIOUR IN THE RAT. M. Hong*, B.J. Chiasson, K.M.A. Murphy and H.A. Robertson, Department of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

Previous work demonstrated that a single intrastriatal infusion of fully thio substituted antisense oligonucleotides (s-ODNs) targeted to *c-fos* mRNA attenuates striatal *c-fos* expression and results in ipsiversive rotational behaviour in rats challenged with amphetamine. However, multiple infusions of these s-ODNs into the brain were found to produce neurotoxic damage. Although a reduction in the number of thio substitutions may minimize neurotoxicity, few studies have examined the effectiveness of partially substituted antisense s-ODNs *in vivo*. In the present study, the effects of single and double end-capped s-ODNs targeted to *c-fos* mRNA on amphetamine-induced striatal *c-fos* expression and rotational behaviour were examined in rats. Intrastriatal infusions of a single end-capped s-ODN (2 nmol) 1, 2.5 or 5 but not 10 hours prior to amphetamine challenge (5 mg/kg i.p.) attenuated striatal *c-fos* expression and resulted in ipsiversive rotational behaviour in a time-dependent manner. Similarly, infusions of a single or double end-capped s-ODN (0.5 - 6 nmol) 2.5 hours prior to amphetamine challenge resulted in similar effects in a dose-dependent manner. However, at the higher doses of these antisense s-ODNs (4 - 6 nmol) some animals demonstrated seizure activity and increased *c-fos* expression was observed in the cortex, thalamus and lateral striatum in addition to an atypical pattern of amphetamine-induced *c-fos* expression in the contralateral striatum. Thus, the present data suggest that infusion of low dose single or double end-capped s-ODN is effective in attenuating striatal *c-fos* expression and inducing ipsiversive rotational behaviour in amphetamine challenged rats while at higher doses both s-ODNs lead to seizure activity in some animals.

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817.11

ANTISENSE OLIGODEOXYNUCLEOTIDES: COMPARISON OF THE UPTAKE AND LOCALIZATION OF PARTIAL AND FULL PHOSPHOROTHIOATE DERIVITIVES IN BRAIN. H.A. Robertson*, Lab. Molecular Neurobiology, Dept. Pharmacology, Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4H7

Antisense oligodeoxynucleotides (ODNs) represent a powerful new technique for manipulating gene expression *in vivo*. Endo- and exonucleases present in tissues rapidly degrade antisense ODNs thus limiting their effectiveness and in general, most people have worked with antisense ODNs where the phosphodiester bond is modified to a phosphorothioate bond. However, we and others have found fully-phosphorothioate derivitized ODNs to be toxic when administered chronically. In an attempt to reduce toxicity, we have studied antisense ODNs which have only a single phosphodiester linkage at the 5' and 3' ends of the ODN modified to phosphorothioate derivatives (chimeric ODNs). Behaviourally, we observed that the onset of activity for such chimeric antisense ODNs directed against *c-fos* was much quicker than for the fully phosphorothioated ODNs. This has been examined using FITC labelled antisense ODNs. Rats were injected directly into the striatum with 2 μl of distilled water containing 1 nmol of either a chimeric antisense ODN to *c-fos* or a fully phosphorothioated antisense ODN to *c-fos*. At various times up to 24 hr after injection, the animals were perfused, sections cut and the uptake and localization of the antisense ODN was followed using fluorescence microscopy. The chimeric antisense ODN to *c-fos* appeared to diffuse more rapidly in striatum and was completely gone at 24 hr; the fully derivitized ODN was still present at 24 hr. Interestingly, both the chimeric and the fully phosphorothioated ODNs appear to be selectively taken up by neurons in globus pallidus. (supported by the MRC of Canada and SmithKline Beecham Pharma Inc).

817.12

INDICES OF ON-LINE AND PREPROGRAMMED CONTROL OF OPERANT RESPONSE FORCE IN RATS. A. B. Slifkin* and J. Brener, Dept. of Psychology, SUNY, Stony Brook, NY 11794-2500.

Research on human subjects has shown that control of Peak response Force (PF) by Time to Peak Force (TPF) reflects the operation of feedback (FB) processes, whereas control of PF by the Rate of Rise of Force (dF/dt) reflects the operation of feedforward (FF) processes. The current study tested whether TPF and dF/dt have the same relationships to FB and FF control of response force in rats. Knowledge-of-Results (KR) (Immediate or Terminal) and Force Contingency [LOW (2 g) or HIGH (18 g)] were examined in a 2 x 2 between-subjects design. Under conditions of Immediate KR, food reward delivery occurred at the moment during response execution that the imposed force requirement was achieved. This afforded the opportunity for response force to be regulated by the available external FB. It was anticipated that if rats make use of the Immediate KR in controlling PF, then HIGH-LOW differences in PF would be determined by HIGH-LOW differences in TPF. Under Terminal KR, use of information about response correctness during response execution was prevented since food for responses meeting the imposed force requirement was delivered at response end. Under these conditions therefore, rats were forced to rely on FF control and it was anticipated that HIGH-LOW differences in PF would be determined by HIGH-LOW differences in dF/dt. Both expectations were confirmed indicating that in rats, as in other species, modulation of PF by TPF reflects FB control whereas modulation of PF by dF/dt reflects FF control.

817.13

CONTROL OF RESPONSE FORCE IN RATS: EFFECT OF BILATERAL PARTIAL 6-OHDA NIGRAL LESIONS AND DOPAMINERGIC DRUGS. J. Brener*, R.E. Strecker†, and X. Liu, Depts of Psychology and †Psychiatry, SUNY, Stony Brook, NY 11794-2500.

The cardinal signs of Parkinson's disease (PD) include bradykinesia and tremor. In addition, PD patients show deficits in isometric force production and the temporal features of individual movements. To understand the mechanism of these behavioral deficits we examined changes in both the force and timing of individual isometric responses in a rat model of PD (Liu et al., this volume). Hungry rats were trained to apply force to a central beam for 2 s. Following beam release, visual stimuli were presented to signal which of two other beams (left or right) should be pressed. A sugar water reward was made available if the left beam was pressed with low force (< 3g), or if the right beam was pressed with high force (>50g). On each session rats made approximately 80 long-duration responses, 100 high-force responses and 100 low-force responses.

Bilateral, partial 6-OHDA nigral lesions resulted in significant increases in the numbers of low-force and high-force errors. These errors were entirely due to loss of control over the rate of rise of force (df/dt): Response timing was uninfluenced either during responses that demanded force control or during responses that required duration control. These results are attributed to a deficiency in the programming of response force rather than to deficiencies in the on-line regulation of motor output (see Slifkin & Brener, this volume). Related results from pre-lesion drug probes with apomorphine, haloperidol and amphetamine will also be reported.

817.14

DEFICITS IN RESPONSE SELECTION PRODUCED BY BILATERAL PARTIAL 6-OHDA NIGRAL LESIONS AND DOPAMINERGIC DRUGS. X. Liu*, R.E. Strecker†, and J. Brener, Depts of Psychology and †Psychiatry, SUNY, Stony Brook, NY 11794-2500.

A sensitive behavioral test was developed to track changes in sensorimotor capacity produced by partial nigral lesions in rats. Hungry rats were trained to apply force to a central beam for 2 s. Following beam release, visual stimuli were presented to signal which of two other beams (left or right) should be pressed. A sugar water reward was made available if the left beam was pressed with low force (< 3g), or if the right beam was pressed with high force (>50g). Following collection of the reward, stimuli instructed the animal either to repeat the last response or to start a new sequence by pressing the central beam. The task was designed to measure simple and choice reaction times as well as to record various sorts of response errors.

After bilateral 6-OHDA nigral lesions, which resulted in a loss of DA neurons in substantia nigra and 50-80% forebrain DA depletions, RT lengthened as an inverse function of stimulus probability. Thus, data showed that simple RTs lengthened least and RTs to the most improbable stimuli lengthened most. This is consistent with the interpretation that the timing of basic sensory and motor processes were uninfluenced by the lesions, but that the processes of response selection were profoundly slowed. Error data relevant to this interpretation as well as pre-lesion data obtained during drug probes [apomorphine (.01, .03, .1 mg/kg, sc), amphetamine (.03, .1, .3 mg/kg, ip) and haloperidol(.01, .03, .1 mg/kg, ip] will be reported.

817.15

BASAL GANGLIA AND CEREBELLAR INPUTS TO SUPERIOR COLLICULUS CONTROL HEAD AND MOUTH MOVEMENTS ELICITED BY FORMALIN INJECTIONS INTO THE RAT HIND PAW. S. Wang, N. Wood, M. Simkins and P. Redgrave*. Dept. Psychology, University of Sheffield, Sheffield, S10 2TP, U.K.

The dorsolateral substantia nigra reticulata (DL- SNR) projects directly to the parvicellular medullary reticular formation (PCRt), an oral premotor region, and to the lateral intermediate layers of the superior colliculus (L-SC), which participate in head control. Both the L-SC and the PCRt also receive direct projections from the lateral deep cerebellar nucleus (L-DCN). The purpose of the present study was first, to characterise in more detail anatomical connectivity from DL-SNR and L-DCN to the L-SC and PCRt, and second, to establish the role of this circuitry in a task requiring precise co-ordination of the head and mouth.

Multiple retrograde and anterograde transport of tracers was used to investigate neural connectivity at the light microscopic level. The main findings were: (i) In rostral DL-SNR a high proportion (>70%) of retrogradely labelled cells were double labelled following some of the injections of fluorescent dyes into L-SC and PCRt. (ii) In L-DCN segregated populations of single labelled cells were found following similar injections of fluorescent tracers into L-SC and PCRt. (iii) Simultaneous injections of PHA-L into DL-SNR and biotinylated dextran into L-DCN produced intermingling patterns of anterograde terminal label in L-SC which surrounded cells retrogradely labelled with cholera toxin B injected into PCRt.

An injection of 50µl of 4% Formalin into the hind paw of a rat produces a reliable biphasic response in which the animal turns to lick and bite the injected paw. This response was suppressed in a dose-dependent manner by bilateral injections of the GABA agonist muscimol into the L-SC (12.5-50ng: 0.5µl/side). Interestingly, the animals' attention and activity were redirected from lower to upper regions of space when doses of muscimol >20 ng were injected into L-SC.

These data suggest that: i) premotor circuits controlling the head and mouth receive converging input from both basal ganglia and cerebellum; and (ii) the integrity of this circuitry is essential for effective performance of a task requiring movements of the head and mouth. *Supported by Wellcome Trust grant 038011/Z/93 to PR*

CEREBELLUM: GENETIC MODELS

818.1

INHIBITION OF DEEP CEREBELLAR NUCLEI NEURONS IN DYSTONIC RATS. J.J. Fu* and J. F. Lorden. Dept. of Psychology, Univ. Alabama at Birmingham, Birmingham, AL 35294

In the genetically dystonic (dt) rat, a model for torsion dystonia, the deep cerebellar nuclei (DCN) have been identfied as a site of abnormality. This study used extracellular single unit recording and microiontophoresis to test the sensitivity of DCN neurons to inhibitory input. Under urethane anesthesia, GABA (0.1M, pH 4.5), taurine (0.5M, pH 3.0), and bicuculline (5mM, pH 3.0), the GABA antagonist, were applied to DCN neurons in dt rats and unaffected littermates, aged 18-25 days. In littermates, all DCN neurons tested (n=17) were inhibited by GABA. Inhibition was defined as a reduction in firing rate of at least 50% at ejection currents of 90 nA or less. In the dt rats, only 79% of the neurons tested (n=24) responded. Unresponsive cells were located mainly in the n. interpositus. In responsive cells, reduced sensitivity in comparison with littermates was noted in the lateral nucleus. In littermates, 56% of the neurons tested (n=18) were inhibited by taurine. In dt rats, however, only 23% of the cells tested (n=22) responded. All cells tested showed increases in firing rate to application of bicuculline. These results are consistent with studies showing decreased binding of ^{3}H-muscimol in the DCN of dt rats and indicate reduced inhibitory control in the dt DCN. (Supported in part by the Dystonia Medical Research Foundation.)

818.2

INCREASED SOMATAL PARVALBUMIN AND GLYCINE IMMUNOREACTIVITY IN THE CEREBELLAR TARGETS OF PURKINJE CELL DEGENERATION MUTANTS. J. Bäurle and U. Grüsser-Cornehls. (SPON: European Neuroscience Association). Dept. Physiol., Freie Univ. Berlin, 14195 Berlin, Germany

The complete loss of Purkinje cells (PC) in Purkinje cell degeneration (PCD) mutant mice removes the major inhibitory input to the deep cerebellar (DCN) and vestibular nuclei (VN). Neuronal activity, however, is not enhanced in the VN, suggesting compensatory reactions of other inhibitory neurons, but the total number of GABAergic cells is not increased and the density of GABAergic terminals is greatly reduced compared to wildtypes. In the present study we show that the number of Parvalbumin-immunopositive (Parv+) somata, which are absent in the DCN and at a very low level in the VN of normal wildtypes, increases massively in these nuclei of PCD mutants. This increase is closely correlated temporally and spatially with the PC loss. In addition, Glycine-immunopositive (Gly+) neurons are drastically more packed in the DCN of PCD, and even after correcting for nuclear shrinkage (≈50%) the total number of Gly+ cells remains significantly higher by 1/3 compared to wildtypes. Moreover, the size and density of Gly+ terminals contacting DCN neurons are increased in PCD. Double labeling shows that Parv, which often colocalizes with GABA, is highly localized in small and medium-sized Gly+ neurons of the DCN and VN in PCD mutants. Taken together, the increase in Parv+ neurons that mainly colocalize with Gly and in terminal size and density of Gly+ small and medium-sized neurons reflect most probably an enhanced inhibitory activity and, as these are presynaptic to the large output neurons, a reduction in turn of the activity of the latter. This mechanism is likely to compensate for the PC loss and contribute to the mild motor deficit in PCD mutants, as a somatal Parv increase can also be evoked in single subdivisions of the DCN and VN by discrete cerebellar lesions in wildtypes, and in Weaver mutants where motor impairment is improved by this operation.

818.3

c-fos EXPRESSION IN THE CEREBELLUM OF *SHAKER* MUTANT RATS WITH HEREDITARY PURKINJE CELL DEGENERATION. D.L. Tolbert*, P. Sharp, M. LaRegina and P. McCulloch. Depts. Anat. &. Neurobiol. and Surg. Saint Louis Univ. and Div. Comp. Med. Washington Univ, St. Louis, MO. 63104

Three month old *Shaker* mutant rats are characterized by coincident degeneration of lobule I-IX Purkinje cells and motor ataxia. Immunochemistry was used to assess c-fos activity in the cerebella from *Shaker* mutant and normal rats. Fos immunoreactivity was observed in the cortex, white matter, and deep nuclei in mutant rats. In the cortex clusters of Fos immunopositive cells were located in the granule cell layer of lobules I-IX. There were no immunopositive cells located in other cortical layers. Subjacent to the clusters of Fos positive cells in the granule cell layer Fos immunoreactive cells were frequently present in the white matter. In other areas clusters of positive cells were located in the white matter at the base of folia and lobules. Fos immunoreactive cells were present throughout the cerebellar nuclei. These cells were more numerous and grouped together in regions deafferented of inhibitory corticonuclear input. Comparable patterns of Fos immunoreactivity were not observed in normal cerebella. These findings of increased cerebellar activity in *Shaker* mutant rats could be the basis for the ataxia and tremor seen in these animals. (Supported by NIH grants RR07013 and NS20227).

818.4

DEVELOPMENT AND QUANTIFICATION OF ATAXIC GAIT IN THE SHAKER MUTANT RAT. B.R. Clark*, A.J.Bastian, T. Weatherspoon, and W.T. Thach. Program in Physical Therapy, Dept. of Anatomy, Wash. Univ. Sch. of Med., St. Louis, MO. 63108, and McNair Entry Fellowship Program, Knox College, Galesburg, ILL. 61401.

The shaker mutant rat spontaneously demonstrates Purkinje cell and inferior olive degeneration (LaRegina et al., Lab. Animal Sci. 42: 19-26, 1992). Clinically shaker rats are classified as strong shakers or mild shakers depending on the presence or absence of tremor. We were interested in determining whether gait deficits in the strong shaker mutants correlated with the described progression of Purkinje cell degeneration (Tolbert et al., J. Comp. Neurol., in press). At regular intervals between 1-6 months of age, we evaluated gait by having normal and shaker rats traverse a clear plexiglass tunnel. Hindfeet were marked with black ink and forefeet were marked with red ink for footprint identification. Measurements of stride length, step length, and stride width were made. Spatial distribution of hindlimb footprints relative to ipsilateral forelimb footprints were measured. Landmarks on the hip, scapulae, wrist, and ankle were marked with black ink. Left forelimb and hindlimb kinematics were videotaped and analyzed using Peak Performance.

At 1 month of age, shaker gait appeared normal. By 3-4 months of age, walking track analysis of shaker gait revealed decreased step and stride length, increased hindlimb stride width, and increased variation of hindlimb placement relative to ipsilateral forelimb placement. Videotape analysis of kinematic gait abnormalities showed forelimb high stepping, highly varied ankle path during hindlimb swing phase, larger and more variable hip displacement, increased time spent in stance phase, and disturbed temporal and spatial pattern of footfalls. Hildebrand plots revealed a lack of temporal synchrony in both the lateral sequence and diagonal couplet that characterizes normal rat gait. At 6 months of age these deficits are more pronounced. In addition, the rats fell more frequently and took extra footfalls uncoupled with footfalls of the ipsilateral limb. These results are consistent with the described time course and extent of Purkinje cell degeneration in shaker rats (Tolbert, ibid). (Supported by the Program in P.T.)

818.5

INFERIOR OLIVARY NEURON NUMBER IN DEVELOPING NORMAL AND *LURCHER* MICE. J. A. Heckroth*. Department of Anatomy and Neurobiology, St. Louis University Medical School, St. Louis, MO 63104-1028.

The number of neurons which comprise the inferior olivary complex has been estimated in wild-type and *lurcher* mice at 10, 20, and 30 days of age. Current results reflect neuronal number estimates in three mice of each genotype at each age. Serial 50μm frozen sections were stained with cresyl violet and analyzed using a Neurolucida® system. Boundaries were drawn around each of four olivary subdivisions (caudal and rostral medial accessory olives, dorsal accessory olive, and principal olive) in every other section, and these outlines were used by Neurolucida® to generate volume estimates of each subdivision. Neuron density in each of the subdivisions was estimated by counting the number of neuronal nuclei within a randomly positioned 125,000 μm^3 cube of tissue in each section. Average density values were multiplied by the volume to provide neuronal number estimates of each subdivision. The number of neurons in the wild-type inferior olive remains relatively constant (about 13,000) over the period examined. The number of *lurcher* olivary neurons is already reduced by about 30% at 10 days of age, and falls to about 30% of normal by 30 days. Although a previous study demonstrated homogenous olivary atrophy in adult *lurcher* mice, the present results suggest a differential rate of loss from the olivary subdivisions. Most notably, the current data suggest a relative sparing of neurons in the caudal medial accessory olive during the period investigated. This work was supported by NINDS grant NS33969.

818.6

COMPENSATORY MECHANISMS IN WEAVER MUTANT MICE AFTER LESIONS OF THE CEREBELLAR VERMIS. C. Grüsser, J. Bäurle and U. Grüsser-Cornehls. (SPON: EUROPEAN NEUROSCIENCE ASSOCIATION). Dept. Physiol., Freie Univ. Berlin, 14195 Berlin, Germany

Recent investigations in Weaver mutant mice, which suffer from an almost complete loss of cerebellar granule cells, a portion of the Purkinje-cells (PCs, mostly in the anterior vermis) and a degeneration of dopaminergic cells in the substantia nigra, have shown that ablation of the cerebellum improved the poor motor performance (severe ataxia, instability of gait, posture and balance) significantly. Electrophysiological experiments revealed a disorganized and faulty output of the surviving PCs and a disinhibition of type i vestibular nuclei cells. On the basis of these findings we removed the anterior vermis in young animals. Their motor performance was observed in an arena divided into 10 x 10 cm squares and the number of tumblings counted within a 5 min walking period in relation to the squares traversed (t/k). T/k improved by 77% in relation to the preoperative values and manoevering on a slanted wire and wooden bench recovered dramatically.

Immunocytochemical investigations revealed an increase in GABA- and Parvalbumin-positive soma labelling of small and medium-sized neurons in the deep cerebellar (Fastigius) and Deiter's nuclei, both projection sites of the anterior vermis. Parvalbumin is a Ca^{2+}-binding protein and thought to indicate high activity levels of the respective neurons. Since a large portion of the small and medium-sized neurons are inhibitory, a tentative assumption is that by removing the total population of PCs in a restricted cerebellar area, a shift in the inhibitory power occurs, leading probably to the better motor compensation in the operated Weaver. The same mechanism is observed in PCD mice, which have an almost complete lack of PCs and in normal wildtypes in which again a portion of the cerebellum was removed.

818.7

GROOMING IN WEAVER MUTANT MICE. R. Lalonde* and C. Strazielle. Université de NANCY I et Université de Montréal, Centre de recherche en sciences neurologiques, Université de Montréal, Montréal (Qc) Canada H3C 3J7

Since electrical stimulation of the medial parts of the cerebellum (vermis and fastigial nucleus) elicits grooming in normal rats, we wished to determine to what extent cerebellar atrophy affects the grooming response. Weaver mutant mice, characterized by degeneration of cerebellar atrophy affects the grooming response. Weaver mutant mice, characterized by degeneration of cerebellar granule cells and midbrain dopamine neurons, were compared to normal mice during self-grooming after water immersion. There were no intergroup differences for grooming episodes, grooming sequences, and grooming episodes per sequence. One grooming component appeared less frequently in weaver mutants: face washing and one more frequently: abdomen licking. Normal mice began a sequence by grooming an anterior body part on 68.9% of occasions, whereas this pattern was reduced (32.5%) in weavers.

Funded by NSERC (Canada).

818.8

AN ELECTRON MICROSCOPIC ANALYSIS OF NORADRENERGIC INNERVATION OF THE WEAVER CEREBELLUM. L.C. Abbott* and C. Sotelo. Dept. of Vet. Anat. & Public Hlth., Texas A&M Univ., College Station, TX 77843, USA and INSERM U.106, 47 Blvd. de l'Hopital, 75651 Paris, FRANCE

Although monoaminergic (MAergic) systems can form classical synaptic junctions in many central regions, the vast majority of MAergic axon terminals are "non-junctional", which correlates with the paracrine action exerted by these systems. To assess the influence of the cellular environment on synaptic modeling of cerebellar MAergic axons, noradrenergic innervation of the wild type mouse cerebellum was compared with that of the weaver (agranular) cerebellum. Noradrenergic axons were ultrastructurally identified using immunocytochemistry for tyrosine hydroxylase (TH), with an antibody provided by R. Reinhardt. In wild type and weaver cerebella, almost all TH+ varicosities were non-junctional and their density was much greater in weaver than in wild type cerebella. From large samples of TH+ varicosities, neuronal elements apposed to noradrenergic axon terminals were identified and quantified. 80% of the TH+ varicosities were apposed to dendritic profiles, of which about half belonged to Purkinje cell dendrites. 60% of the TH+ varicosities were apposed to other axon terminals. In wild type mice, 45% of the dendritic profiles apposed to TH+ varicosities were synaptically contacted by apposed TH- axon terminals, whereas in the weaver mouse this percentage decreased to 22%.

These results emphasize that cerebellar noradrenergic innervation is almost exclusively of the non-junctional type, and that Purkinje cells do not appear to be the only target of this innervation. Granule cell absence does not change the fate of the noradrenergic innervation, because it does not evolve from a mostly non-junctional into a junctional input. This is in contrast to the report for serotonin axon terminals in a rat phenocopy of the weaver mutation (Beaudet and Sotelo, Brain Res. 206:305, 1981).

CONTROL OF POSTURE AND MOVEMENT: HUMAN LOCOMOTION

819.1

SYNCHRONIZATION EFFECTS OF AUDITORY RHYTHM ON GAIT IN HEALTHY ELDERLY AND PARKINSON'S DISEASE PATIENTS. M.H.Thaut*[1], G.C.McIntosh[1], S.H.Brown[2], R.R.Rice[3], C.A.Mezza[1], R.A. Miller[1]. Center for Res. in NeuroRehab., Colorado State Univ.[1]; Center for Human Motor Res., Univ. Michigan[2]; Poudre Valley Hospital[3]; Fort Collins CO 80523.

The effect of auditory rhythm on stride timing in Parkinson's disease (PD) was studied in a frequency entrainment design. Twenty-one PD patients on dopaminergic medication (ON), 10 PD patients off medication (OFF), and 10 healthy elderly (HE) subjects completed 4 tasks: (1) 30 m baseline walking at preferred speed; (2) walking with rhythmic cue matched to their baseline cadence; (3) walking with rhythmic cue 10% faster than baseline; (4) walking with rhythm faded.

Rhythmic signal frequency entrained gait cadence across all frequencies in all subjects except 2 ON-PD patients exhibiting continuous step-to-cue phase drift. Rhythmic cuing decreased variability of stride intervals (IRI) in the OFF-PD group by 62%, and between 10 and 30% in the HE and ON-PD group. Rhythmic stimulus interval (ISI) and IRI were closely matched across all frequencies. Offset values ranged from 8 to 40 ms. OFF-PD patients had larger synchronization errors (SE) (matched cuing: 125 ms; faster cuing: 142 ms) than ON-PD patients (75 ms; 98 ms) and HE subjects (72 ms; 92 ms). Phase angle transformations of SE to normalize for ISI differences showed the same results. SE variability was similar between OFF-PD and ON-PD groups (40.5 vs 47.3 ms) and increased slightly with faster cuing in both groups (45.9 vs 48.2 ms). We conclude that timekeeper entrainment occurred in the HE and both PD groups, however with larger and more variable synchronization offsets in the OFF-PD group.

819.2

FORCE SYMMETRY FEEDBACK TRAINING ON THE BICYCLE IN PATIENTS WITH UNILATERAL CEREBROVASCULAR ACCIDENTS. K.L. Perell,[1] R.J. Gregor,*[2] A.M.E. Scremin.[1] [1]PM&RS, WLA VAMC, LA, CA 90073 & [2]Georgia Tech, Atlanta, GA 30322.

The purpose of this study was to examine the ability of subjects with unilateral cerebrovascular accidents (CVAs) to utilize kinetic feedback to modify pedaling techniques. The relationship between performance and neurophysiological parameters such as sensation, propriception, kinesthesia, and cognition were explored. Eight male subjects with unilateral CVAs were randomly assigned to two groups. The bicycle apparatus was a recumbent bicycle with pedals capable of measuring normal and tangential components of the applied force. Training consisted of twelve one-minute trials with one minute rest between trials, three times a week for four weeks. Feedback consisted of visual and verbal feedback regarding patterns approximating the effective force bilaterally after each trial during the rest periods. The no-feedback (NF) group received no feedback. No feedback was given during the pre/post testing sessions. Effective pedaling was determined by the amount of positive crank work involved/noninvolved (I/N) leg ratio. The NF group showed improvement in this parameter, but the FB group did not. Possible explanations for the lack of improvement in the FB group are: 1) the cyclical nature of cycling allowed for natural patterns to develop without feedback; 2) with feedback, frequent trial-to-trial changes interfered with the development of natural patterns; 3) the feedback may have been too complicated for this population; and 4) development of error-detection mechanisms by integrating the visual feedback and kinesthetic variables was limited by the compromised learning and integrating abilities of these subjects. Further, subjects with subcortical lesions tended to perform better than those with cortical lesions. In the three most important effective pedaling parameters, the majority of the subjects who had I/N ratios closer to 1.0 (complete symmetry) had subcortical lesions.

819.3

THE EFFECTS OF CHEMOTHERAPY ON GAIT PATTERNS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA. V.Galea*, C.Mark, J.A.Halton and M.R.Pierrynowski. Human Movement Lab., McMaster Univ., Hamilton, ON, Canada L8N 3Z5.

The majority of children receiving treatment for acute lymphoblastic leukemia (ALL) develop abnormalities of gait, reduced weight bearing and, in severe cases, wheelchair confinement (Halton, et al, 1993). Children with ALL admitted to the McMaster University Medical Centre are treated on the Dana Farber Cancer Institute Protocol, having a prospective of cure approximating 80%. Upon presentation, patients are categorized as either high or standard-risk of relapse (HR or SR) based on (primarily) total white blood cell count and age. Patients in the HR category receive 3X the dosage of corticosteroids (Dexamethasone) than those in the SR category and are cranially irradiated in addition to receiving the SR consolidation and maintenance protocol. SR males receive cranial irradiation but the standard steroid dose.

Complete, kinetic gait analyses were performed on 21 patients who were at various stages of the 24 month treatment protocol. In addition, total serum protein, creatine phoshokinase, serum 3-methylhistidine and hydroxyproline levels were monitored in an effort to assess myofibrillar and collagen degradation secondary to administration of Dexamethasone. The HR females (N=6) were the most severely affected , with the maximum hip joint power at toe-off showing a definite decline as treatment progressed. Those least affected were the SR males (N=7) whose hip joint powers at toe-off did not show a decline as treatement progressed. . SR females (N=5) and HR males (N=3) showed progressive declines in hip powers but to a much lessor extent then the HR females.. Serum 3-methylhistidine and hydroxyproline levels correlated well with maximum hip powers (r=0.748 and 0.891, respectively). Knee and ankle joint powers at toe-off were not affected to the same extent. These findings suggest that the observed gait abnormalities in these children could be attributed to a corticosteroid induced myopathy affecting the large ,proximal lower limb muscles.

819.4

EFFECT OF PARESIS ON GAIT INITIATION AFTER STROKE. F. Malouin*, C.L. Richards, F. Dumas, C. Bonneau, F. Comeau and S. Lavoie. Lab. Neurobiology and Physiotherapy Dept., Laval Univ., Quebec City, PQ, G1J 1Z4, Canada.

The initiation of gait is controlled centrally by a motor program (Crenna & Frigo 1991) that releases preparatory adjustments (PAs) to propulse the body forward and toward the stance limb. The aim of this study was to determine if PAs are preserved after a stroke. The activations of soleus (S), tibialis anterior (TA), and the ground reaction forces were recorded bilaterally in nine subjects (mean of 56 yrs) who had sustained a first stroke. Subjects initiated gait under two conditions: 1) with the paretic limb as the swing limb (PSW) or 2) with the non paretic limb as the swing limb (NPSW) while they stood with one foot on each of two force platforms (AMTI). The latency of PAs, and peak values of the centre of pressure (CP), normalized to the length of the base of support, were compared between conditions and to results from a control group (n=6) walking slowly. The two paretic subjects with highest gait velocity (99, 104 cm/s) displayed characteristics PAs (bilateral TA pre-activation, S inhibition, and displacement of the CP backward and to the swing limb) for the two conditions, but the magnitude and timing of the PAs differed from controls. In the other subjects with a slower gait (28-69 cm/s) only the TA from the NP limb was pre-activated in either conditions. The TA from the NP limb was also activated first whether gait was initiated with the PSW (245 ± 70 ms) or the NPSW (216 ± 98 ms) limb. In addition to this fixed order of recruitment, the latency of TA was longer than controls (140 ± 24 ms) suggesting a bilateral involvement in the planning process of the gait initiation program after stroke. While controls had displayed a nearly symmetrical backward shift (peak CPx) under the swing (12.4%) and the stance (14.2%) limbs, patients had smaller CPx on the P side (4% and 8.8%) compared to the NP side (8.8% and 10.9%) for conditions 1 and 2, respectively. Present results suggest that strategies involving the NP side compensate for impaired feedforward control of gait initiation after stroke (Supported by NHRDP).

819.5

EFFECTS OF INCREASED SPEED AND WORKLOAD ON THE CONTROL OF MOVEMENT IN PERSONS WITH HEMIPLEGIA. D.A. Brown*, S.A. Kautz, and C.A. Dairaghi. Motor Control Physiology Lab. Rehabilitation Research and Development Center at the Palo Alto Veterans Affairs Medical Center (153), Palo Alto, CA 94304 *Funded in part by Foundation for Physical Therapy, Inc.*

Controversy surrounds the once-widely accepted theory that undue exertion during exercise should be avoided because it may exacerbate impaired motor performance in spastic hemiplegia. We hypothesized that the motor performance measured during pedaling in hemiplegia is related to the level of exertion associated with workload and speed. We examined specific measures of motor performance during pedaling for a relatively homogeneous population of hemiplegic stroke patients and compared these with a group of age-matched, neurologically-normal individuals. We measured pedal force output and EMG activity in seven muscles at combinations of five workloads (0, 40, 80, 120, and 160 Joules) and three speeds (25, 40, and 55 rpm). Results demonstrated that stroke patients responded to increased workloads by increasing the force output of both the plegic and nonplegic limbs without increasing the amount of negative work done by joint torques. This result is also accompanied by increased EMG activity in the power generating muscles of both limbs. Interestingly, subjects with poor motor recovery showed better motor performance at lower speeds. We conclude that, during hemiplegic exercise, challenging workloads can enhance motor output without impairing motor performance. However, exercise at challenging movement speeds should be approached with caution.

819.6

THE DYNAMICS OF HUMAN LOCOMOTION: HYSTERESIS AT THE WALK-RUN TRANSITION. F. J. Diedrich* and W. H. Warren, Jr. Department of Cognitive and Linguistic Sciences, Brown University, Providence, RI 02912

Why do humans switch from walking to running as speed increases? According to the dynamic theory, the shift between gaits behaves as a bifurcation between two attractors (Diedrich & Warren, 1995). This theory predicts that the transition should exhibit hysteresis, which is a tendency for the walk to run transition to be made at a higher speed than the run to walk transition. Strong evidence for hysteresis has been found in experiments in which speed was varied in a step-wise manner through the transition region (e.g., Hreljac, 1993). In contrast, in trials in which speed was continuously varied, results concerning hysteresis have been mixed (Diedrich & Warren, 1995). Therefore, in order to clarify these findings, six participants performed transition trials in which the time scale of the change in the control parameter was varied. Specifically, transition trials were performed in the following conditions: 1) speed was continuously varied, 2) speed was changed in steps of 10 s, and 3) speed was changed in steps of 20 s. These trials were analyzed with respect to the direction of the transition, thus resulting in a more complete evaluation of hysteresis.

819.7

WALKING AS INFLUENCED BY DIMENSIONAL CHANGES IN ENVIRONMENTAL OBSTACLES. J. R. Higgins*, B. C. Bennett, J. W. Doyle, S. Higgins. Department of Kinesiology, San Francisco State University, San Francisco, CA 94132

Increasingly, descriptions of the mechanisms of motor control are being cast in a dynamical systems framework. From the dynamical systems perspective walking can be considered an attractor state (a stable pattern) for locomotion. Perturbations of a stable attractor can illuminate the principles and processes that contribute to shifts and stabilities in movement pattern. Subjects walking a linear path in their preferred rate and style, were perturbed by having to traverse an obstacle of various dimensions placed in the path. We looked for phase shifts in the temporal and kinematic organization of the movement and in the strategies chosen to traverse the obstacle. The level platform was 20 cm high. The width of the platform, the critical parameter for the designed task, varied from 1 cm to 75 cm, a range that at times either compels stepping over or stepping upon the platform to traverse it, or that affords either strategy. The width of the platform was varied in random order; increasing to decreasing order; and, decreasing to increasing order. Analysis of the movement strategies and kinematics of the gait in this motor task revealed the presence of hysteresis. That is, in regions where the width of the platform affords the subject either stepping upon or over to traverse it, the individual's recent history determines which movement solution will be chosen and how selected temporal and kinematic variables vary to support these strategies. In addition, the kinematic analysis of the subjects' gait revealed systematic adaptation to the direction of change in platform dimension. These data support the existence of a region of bistability between two stable attractor states in which the individual may opt for either state. The transformations and shifts between the two stable attractor states in a hysteretic fashion, argues strongly for context and experience as integrated in the control of movement.

819.8

ROLE OF BINOCULAR VISION IN OBSTACLE AVOIDANCE DURING LOCOMOTION. A.E. Patla[1]*, M.A. Goodale[2], S. Rietdyk[1], A. Adkin[1], C. Silcher[1], & J.J. Marotta[3] [1]Dept. of Kinesiology, Univ. of Waterloo, Waterloo, Canada, [2]Dept. of Psychology, Univ. of Western Ontario, London, Canada, [3]Neuroscience Program, Univ. of Western Ontario, London, Canada.

We are interested in determining how binocular vision influences the kinematics of obstacle avoidance during locomotion. Young healthy subjects (n=7) were asked to estimate heights of obstacles verbally, and following that subjects (n=6) were required to step over obstacles of three different heights (Low-0.5cm, Medium-18cm, High-38cm) during locomotion under monocular and binocular viewing conditions. Head and lower limb kinematics were monitored using an OPTOTRAK system. Verbal estimation of obstacle height was similar under two viewing conditions, although the variability in slope of the regression line between the estimated and actual height was more variable under monocular viewing. Choice of leading limb was affected by monocular viewing but only for the high obstacle: when the right eye was patched subjects preferred (approx. 2 to 1) to lead with the left limb and vice versa. Leading limb toe clearance was affected by monocular viewing, but only for the high obstacle (15.4 vs 12.7cm). Trailing limb toe clearance was higher for the monocular viewing condition for all three obstacle heights (H-13.6 vs 12.8cm; M-14.3 vs 12.3cm; L-6.1 vs 4.9cm). Viewing strategies differed among subjects. For 3 subjects relative head pitch angle (with respect to the trunk) over the obstacle was affected by monocular viewing: they looked down more for the high obstacle (20.6° vs 16.1°). Maximum pitch angle occurred before the obstacle in the monocular viewing condition and after the obstacle in the binocular viewing condition. Supported by grants from NSERC Canada.

819.9

CONTROL STRATEGIES FOR VISUALLY GUIDED STEPPING

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Human subjects performing a task that requires visual guidance of each step onto irregularly placed 'stepping stones' usually foveate the next target of footfall just before they lift the foot to be repositioned ie towards the end of stance. When asked to negotiate the same walkway without ambient lighting, and with each stone's location indicated by a central light spot (LED), subjects' stepping and eye movements are unchanged. Under conditions of intermittent visual denial, in which all LEDs were temporarily extinguished at irregular intervals, a proportion of steps are affected, but not all, suggesting that uninterrupted on-line visual feedback is not crucially important, even when (as here) each step required visual guidance. In an affected step, the effect is primarily on stance, rather than swing, and is an increase in stance duration, suggesting an effect on planning during stance of the next step (next swing towards the next target) rather than on execution of the ongoing step (current swing). Under 400 and 500 ms denial, there is no effect when the target disappears in the first 100ms of stance; this implies that crucial planning does not take place until near the end of stance of the foot to be repositioned. Accurate saccades, followed by accurate steps, to the next footfall target are often made when it is invisible. This implies that saccade generation might itself be useful even though it does not result in a foveal image ie the neural processing preceding a saccade to a target is used in planning the next step. We propose that a task such as this is executed under central nervous control operating as a feedforward visuomotor control system that is robust during periods of visual denial of up to 800ms. Analysis of eye movements recorded during this experiment (currently underway) should further elucidate the underlying control strategies used in this locomotor task.

819.10

CONTROL OF TIMING OF HEALTHY ADULT GAIT DURING WALKING AT VARYING CADENCES. S. J. Lee*. Department of Physical Therapy, National Yang-Ming Univ. Taipei, Taiwan, ROC.

Schmidt's invariant phasing theory of motor control (1976, 1985, 1988) is a theory of strict central control of timing in which the proportional duration of the kinematic events of a well-learned or an innate movement pattern is invariable while the overall period of time to complete the movement pattern varies. This study attempted to assess the applicability of Schmidt's invariant phasing theory in mature human locomotor movements by determining if consistency exists in the proportional duration of selected 2 hip, 4 knee and 4 ankle kinematic events in the sagittal plane of healthy adult gait while walking at varying cadences. A computerized videotape gait analysis system was employed. Sixteen repeated stride cycles at preferred, fastest, and slowest cadences, respectively, for each of 18 healthy adults (Mean age=27±6 years, 9 males and 9 females) were analyzed.

The results of this study showed a high degree of consistency in the proportional duration of selected kinematic events during the total stride cycle within repeated trials of each of the walking conditions at preferred, fastest, and slowest cadences. This finding indicates that the control of timing of healthy adult gait within a specific walking cadence is essentially central. However, significant differences ($p < .05$) were found in the proportional duration of all selected kinematic events during the total stride cycle among different walking conditions at preferred, fastest, and slowest cadences. This part of findings indicates that the central control of timing of healthy adult gait is not strict at varying cadences. The results of this study, taken as whole, is better interpreted in light of the view of a task-related control mechanism of timing in mature human locomotor movements. It also suggests a need to refine Schmidt's invariant phasing theory.

819.11

SEGMENTAL COORDINATION DURING HOPS USING ONE OR TWO FEET IN CHILDREN AND ADULTS. P.A. McKinley[1]*, C. Assaiante[2], B. Amblard[2], and L. Pelland[1]. McGill University, Sch. of P.O.T., Montreal Quebec H3G 1Y5[1] & UPR Neurobiologie et Mouvements, CNRS, Marseille France[2]

The main purpose of this study was to investigate the development of segmental coordination during single hops using one foot or two feet in children of two ages (5.5-6 and 7-7.5yrs) and adults (n=6/group). Kinematics of the legs, trunk and head, kinetics at takeoff and landing, and EMG of the trunk, lower limb and neck were collected using an integrated motion analysis system (ELITE, BTS). Results were analyzed separately for 3 phases - takeoff, flight and landing - and indicated the following: 1) for head/trunk control, the children differed from adults, but not from each other, while at the lower limb, the three groups used different modes of advancing the body; 2) children use significantly more trunk and head motion to advance the body during bipedal hops than during unipedal hops and as compared to adults; 3) head stabilization in space is phase dependent, while trunk stabilization is phase independent in all subjects. It is suggested that poor timing of, and lack of adequate propulsion during the takeoff phase in children may be responsible for the observed differences. Similarities in head/trunk stabilization in space suggest that the trunk may contribute a stable reference frame from which balance control is organized. Supported in part by NSERC & FRSQ

819.12

MODULATION OF RECIPROCAL INHIBITION DURING WALKING AND VOLUNTARY MOVEMENT IN HUMANS. B. A. Lavoie*, H. Devanne and C. Capaday. Centre de Recherche en Neurobiologie, Université Laval, Québec (Qc) Canada, G1J 1Z4

These experiments were designed to evaluate task dependent control of reciprocal inhibition during natural motor activities. We measured the effect of tibialis anterior (TA) activation on the amplitude of the soleus (SOL) H-reflex during the swing phase of walking (SW), one leg stepping (OLS), phasic TA contraction (PC) and tonic TA contraction (TC). Linear regressions of the mean TA rectified EMG vs. SOL H-reflex amplitude were compared to determine whether slopes or y-intercepts (y-int.) differed significantly between these tasks. During PC and TC, the H-reflex amplitude stayed well above zero at all TA EMG levels. During SW and OLS the amplitude of the SOL H-reflex was reduced to, or near, zero during ankle dorsiflexion at all TA EMG levels. When we compared SW and PC, we found that the slopes were not significantly different but the y-int. were significantly higher for PC. When we compared SW and TC the y-int. were always significantly greater for TC. Capaday et al. [J. Neurophysiol. 64: 607-616, 1990] showed that there was no difference in the amount of reciprocal inhibition acting on the active SOL between voluntary tonic activity and the stance phase of walking. In complement to that study, the present results suggest a strong task dependent modulation of reciprocal inhibition onto the inactive SOL (i.e. when it acts as an antagonist). In summary, there was a difference between SW and PC or TC, but not between SW and OLS. The strong reciprocal inhibition occurring in a task in which the SOL is stretched (SW, OLS, PC) could be due to increased presynaptic inhibition of Ia-afferents onto SOL α-motoneurons, or increased hyperpolarization of SOL α-motoneurons. As for the lack of difference between SW and OLS, this could be due to a fine tuning of reciprocal inhibition in order to ensure clearing of the foot off the ground.

819.13

H REFLEX MODULATION IN TIBIALIS ANTERIOR DURING PASSIVE PEDALLING. J.D. Brooke, W.E. McIlroy*, M. Miklic, W.R. Staines and J.E. Misiaszek. Department of Human Biology and Nutritional Sciences, University of Guelph, Ontario, Canada N1G 2W1

Soleus H reflex gain is strongly modulated, leading to marked attenuation, consequent to passive movement of the human leg. We addressed the lack of information about any other H reflex of the leg with such movement, hypothesizing that attenuation also occurs for tibialis anterior (TA), in antiphase to the firing pattern this muscle shows during active pedalling. With eight subjects, thirty TA H reflexes were collected at four equispaced phase positions with two passive movement velocities (20 and 60 rpm of the pedal crank). M waves indicated stimulus stability. TA was tonically contracted throughout the cycle, at 5- 15% of the EMG for a maximum voluntary contraction. No significant movement-induced attenuation, or phase modulation of the reflex, occurred for either velocity. Mean maximum depression was 19% of controls, at full extension ($p > .05$). This lack of modulation for the TA H reflex contrasts with typical reductions of up to 80% of control for soleus. Such marked contrasts in sensorimotor gain control between the Ia autogenic pathways for muscles of the human leg may indicate functional differences in the spinal reflexes they serve. Supported by NSERC (CANADA).

819.14

H REFLEX MODULATION DURING REVERSE PASSIVE PEDALLING. S.P.Dukelow, J.D.Brooke*, K.B. Adamo, J.Cheng, W.R. Staines and J.E. Misiaszek. Department of Human Biology and Nutritional Sciences, University of Guelph, Ontario, Canada N1G 2W1

The gain of the soleus H reflex attenuates to the rate of passive movement of the human leg. The attenuation is maximal at the end of leg flexion. In this phase, maximum flexion of the hip and knee occur at different positions. We asked, 'Will the gain changes still occur when the direction of movement is reversed, so as to reverse those positions?' In four subjects, reverse passive pedalling movements of the legs were studied at two velocities, 10 and 30 rpm. Ten H reflexes per subject were elicited from TN stimulation in the popliteal fossa, at eight equispaced positions around the cycle. M waves were used as indicators of stimulation stability. For each subject, greatest reflex attenuation occurred in the flexion phase ($p < .01$), but unlike forward movement, it peaked before full flexion of the knee. Movement velocity continued to determine the degree of inhibition ($p < .05$). The reverse versus forward difference in the position of the peak inhibition likely reflects differences in conditioning receptor discharge from hip and knee extensor muscles, due to differing kinematic profiles for the two movements. Supported by NSERC (CANADA).

819.15

MOVEMENT KINEMATICS MODULATE SOMATOSENSORY EVOKED POTENTIAL (SEP) AMPLITUDE. W.R. Staines*, J.D. Brooke and J.E. Misiaszek. Human Neurophys. Lab, Human Biology Dept., University of Guelph, Guelph, ON, Canada, N1G 2W1.

Gating of SEPs becomes more pronounced with increased movement velocity. Also, the gain of the soleus H reflex correlates with the rate of tissue stretch around the knee during passive cycling. We hypothesized that SEP gain would be similarly dependent on the rate of stretch of the knee extensors. SEPs from Cz' and Cz, referenced to Fpz', along with soleus H reflexes were elicited by electrical stimulation of the tibial nerve at the popliteal fossa in 6 subjects. Combinations of rates and ranges of cyclical passive movement of the right leg were used to obtain two sets of three conditions with equivalent estimated rates of stretch of the knee extensors (4 and 16 mm/s). SEPs sampled during passive movement leading to the greater estimated rate of stretch were significantly depressed compared to those sampled during the lower rate of stretch and the stationary controls ($p<0.05$). The maximum attenuation across all conditions was 40% of the stationary controls. There was also a significant rate effect ($p<0.05$) between the conditions with equivalent rates of stretch. Thus, it appears that, at least for mild attenuation, the controls for SEP gain read both movement range and rate, and not simply the rate of extensor muscle stretch. Supported by NSERC (Canada).

819.16

EXPERIMENTAL REMODELLING OF LOCOMOTOR PATHWAY CONTROL. K Weber, WAF Fletcher, C Gordon, G Melvill Jones*, E Block. University of Calgary, Dept. of Clinical Neurosciences, Calgary, Alberta, Canada, T2N 4N1

In a previous study we described an experimental paradigm which adaptively remodelled non-visual control of curvature in the trajectory of forward locomotion in such a way that when trying to walk straight ahead, blindfolded subjects invariably described strongly curved pathways (Gordon et al 1995, EBR 102). In the present study we chose "stepping-in-place" rather than forward locomotion as a means of measuring long-term post-adaptive effects. Six normal subjects participated in 7 trials each, during which they "stepped-in-place" (ie. without turning relative to space) on the center of a horizontal disc which was turning at rates ranging from 11.25 - 90 deg/sec and for durations ranging from 7.5 - 60 min. Following this adaptation procedure, blindfolded subjects attempted "stepping-in-place" on the stationary floor. Results: Post-adaptation all subjects turned relative to space without any perception of rotation, the direction of turn being the same as that of stepping relative to the turning disc. The initial response (up to 20 deg/sec) was linearly related to the velocity of the turning disc (slope=0.41, r=0.79) up to 45 deg/sec after which the response plateaued. The initial response was independent of stimulus duration over the range tested. Two additional response characteristics were: 1) an early (first min) depression (likely due to vestibular stimulation), and 2) a subsequent response decline best fitted by a double exponential curve of short and long time constants on the order of minutes and hours respectively. Presumably this novel, adaptive system normally serves to maintain proper calibrations in the non-visual, ground-based control of locomotor trajectory curvature during forward locomotion.
Supported by the T. Rozsa Foundation, MRC-MT.5630, Alberta Heritage Foundation for Medical Research.

819.17

A SEX DIFFERENCE IN HUMAN TURNING BIAS. L.A. Mead* and E. Hampson. Dept. of Psychology, University of Western Ontario, London, Ontario, Canada N6A 5C2.

Extensive research with rodents has clearly established the existence of individual differences in the preferred direction of spontaneous and induced rotation. Sex differences also exist, in that female rats demonstrate stronger rotational biases than males. In an attempt to generalize these findings to humans, the "human rotometer" has been developed which is capable of measuring spontaneous rotational movement in humans (Bracha et al., 1987). We report here a new method for measuring turning bias in humans which is fast, reliable, and performed under controlled laboratory conditions. Four tapedecks were arranged on tables surrounding an open square area, 3 m across. Every 5 s, one of the tapedecks emitted a 1 s tone, to which subjects responded by approaching the tapedeck and checking a response sheet. Half of the 160 trials were "critical trials" in which the tone was heard from directly behind the subject. The experimenter recorded the direction the subject turned on each trial. Sixty-one right-handed, medication-free subjects (35 males, 26 females) were tested using this paradigm. On average, both males and females demonstrated rightward turning biases on the critical trials, however the tendency to turn to the right was significantly stronger for females. Among 43 subjects who returned for a second session, we found a significant correlation of .77 between the turning biases measured at the two sessions. These findings support the existence of a sex difference in turning bias in humans, and suggest that the rotation task described here is a reliable and valid method of measuring this bias.

819.18

SAGITTAL PLANE HEAD STABILIZATION DURING LOCOMOTION. R.L. Cromwell*. Dept. of Physical Therapy, Temple University, Philadelphia, PA 19140.

The anatomic architecture of the head and neck is one of instability. However, head stability is essential for maintaining gaze during daily activities such as locomotion. In the horizontal plane during locomotion, subjects successfully maintained head stability (Cromwell, Soc. Neurosci. Abstr., 1994). This was accomplished by head with respect to trunk gains < 1 and relative phases $\leq 180°$ at frequencies near 1 Hz. At higher frequencies, gains were now 1 and relative phases were 180° indicating a greater challenge to head stability at these frequencies. A somewhat different picture has emerged in the sagittal plane. Ten subjects walked at their natural cadence while angular velocities of the head and trunk were measured in the sagittal plane. Preliminary results of four subjects showed average peak head velocities of 60.4 °/s toward flexion and 42.1 °/s toward extension. Frequency analyses demonstrated a frequency range of .24 to 8.3 Hz related to this motion. Subjects showed head with respect to trunk gains near 1 and relative phase values of 180° at all frequencies with few exceptions. These frequency and velocity characteristics indicate that the challenge to head stability in the sagittal plane is greater than that of the horizontal plane. Therefore, even at low frequencies in the sagittal plane, head motion perfectly compensates trunk motion in order that head stability is maintained. In the sagittal plane, gravity exerts a greater effect as the head increasingly deviates from vertical. The larger average peak velocity in the flexion direction is an indication of the gravitational effect on head motion in this plane. *Supported by grant DC01125.*

COGNITION XIII

820.1

GLUCOSE ENHANCEMENT OF COGNITIVE PERFORMANCE IN COLLEGE STUDENTS. D.L. Korol*, F.J. Lexcen, M.B. Parent, M.E. Ragozzino, C.A. Manning and P.E. Gold. Univ. Virginia, Charlottesville, VA 22903.

In healthy elderly subjects, Alzheimer's patients and other patient populations, glucose ingestion enhances performance on several tests of memory. Similar findings in young adults, however, have not been evident. Perhaps the tests used to demonstrate facilitation of memory in aged and other populations have lacked the sensitivity or difficulty needed to observe glucose enhancement in young subjects. In the present study, we addressed whether glucose facilitates memory and attention in young adults on more difficult tests. A counterbalanced, crossover design was used, with each subject tested on 2 occasions: after glucose (50 g) and after saccharin ingestion. A series of cognitive tests was administered to assess memory for narrative prose and delayed recognition of faces and words (Squire and Zouzounis, 1993), working memory for words (Salthouse, 1992) and attention (Minnesota clerical number checking test). Significant enhancement of performance by glucose was found on both immediate and delayed recall of the narrative prose passage. Scores on narrative prose and attention tests were positively correlated with peak change in blood glucose after glucose consumption. Facilitation was also seen on completed correct items in the attention test, but only when blood glucose rose to levels greater than 60 mg/dl above baseline. These data suggest that glucose enhances cognitive performance in young subjects when both task load and blood glucose levels are sufficiently increased. In addition, under some conditions, glucose enhances performance on tests that emphasize attention as well as memory. Supported by ONR (N0001489-J-1216), NINDS (NS32914) and NIA (AG07648).

820.2

ODOR IDENTIFICATION IN HUMANS BEFORE AND AFTER RESECTION FROM A TEMPORAL LOBE. M. Jones-Gotman* & R.J. Zatorre. Montreal Neurological Institute and McGill University, Montreal, Quebec, Canada, H3A 2B4.

Mild deficits in odor identification are observed after temporal-lobe resections in patients tested birhinally (Jones-Gotman & Zatorre, *Neuropsychologia* **26**, 387-400, 1988) and monorhinally (Jones-Gotman et al, *Epilepsia* **33**, 135, 1992) using the University of Pennsylvania Smell Identification Test (UPSIT). In this study, we investigated the contribution of possible pre-existing deficits to performance on the UPSIT in 45 epileptic patients tested monorhinally before and after excision from the left or right temporal lobe.

Results showed impairments in both groups before surgery without significant difference between the nostrils, and an overall decline in the left-resection group after surgery. The right-resection group, although still impaired postoperatively, showed a slight gain rather than a loss after surgery.

The postsurgical loss in left-resection patients underlines the strong verbal component in this olfactory task. The pre-operative deficits in both groups show that an epileptic focus in either temporal lobe is sufficient to disrupt normal odor identification. Funded by MRC MT-10314 to MJ-G and RZ.

820.3

THE DEVELOPMENT OF FACE DISCRIMINATION CONTINUES INTO ADULTHOOD: AN ERP STUDY. T.D. Alvarez* and H. J. Neville. UCSD Depts. of Neuroscience and Cognitive Science, La Jolla, Ca 92093.

Clinical, behavioral and electrophysiological studies have led to the proposal that, in adults, specialized mechanisms within the right hemisphere are preferentially involved in the discrimination of faces. Moreover, several studies suggest that adults use these specialized systems in the discrimination of upright but not inverted faces. Comparatively little is known about the developmental time course of the neural and cognitive substrates underlying face discrimination. While some studies suggest that a specialized mechanism for face processing exists even in newborns, behavioral studies have shown that children may not use the same mechanisms as adults in face discrimination tasks. For example, it has been reported that children do not show better performance for upright than for inverted faces, or the left visual field advantage for upright faces seen in adults. These studies have suggested that this adult pattern emerges relatively late in development, sometime in early adolescence.

The present study used behavioral and electrophysiological techniques to examine the development of face discrimination from the age of 9 to adulthood. Event related potentials (ERPs) were recorded from 9, 13, 16 year old, and adult subjects performing a face matching task.

Behavioral findings showed that performance on the task improved with age, and was better for upright than for inverted faces in all age groups. ERP data provided clear evidence of major developmental changes in the processes underlying face discrimination over the age range studied. As in adults, ERPs recorded from children displayed a negativity around 250-450 ms to the second member of a pair of faces. This negativity was greater for mismatched than for matched faces. However, the ERPs of the child groups did not display the hemispheric asymmetry or the difference between upright and inverted faces that was observed in adults. These results suggest that the adult pattern of face discrimination develops gradually, and appears at a much later age than previously believed (> 16 years).

820.4

LATERAL AUDITORY INPUT ALTERS VISUAL PERCEPTION: CROSS-MODAL HEMISPHERE ACTIVATION. Roger A. Drake*, Lisa R. Myers, and Boris A. Marshinin. Dept. of Psychology, Western State College, Gunnison, Colorado 81231, USA and Institute of Psychology, Russian Academy of Sciences, Yaroslavskaia str. 13, Moscow, Russia.

Visual perception was measured by line bisecting. Dividing a horizontal line to one side of the true center indicates a greater relative activation of the contralateral cerebral hemisphere. This perceptual error results from the more-active hemisphere overestimating the length of the line segment in the opposite visual field. Subjects heard audiotaped oral messages about which line to bisect next, in only one ear via full-coverage stereophonic headphones.

The Net measure produced means of -.92 for left-ear input and +.57 for right-ear input, $F(1,26)=4.30$, $p<.05$. The Index measure showed means of -30.77 and +18.00, $F(1,27)=5.41$, $p<.03$.

These results show the power of induced lateral orientation of attention to selectively activate one cerebral hemisphere relative to the other, as previously measured by rCBF (Malamed & Larsen, 1977), cognitive processing (Drake, 1990) EEG (De Toffol et al., 1992), and evoked potentials (Tressoldi & Cusumano, 1992).

820.5

PERCEPTION OF COMPOUND VISUAL STIMULI AND HEMISPHERIC SPECIALIZATION IN HUMANS AND BABOONS. J. Fagot, J. Requin* & C. Deruelle. CNRS, Lab. of Cognitive Neurosciences, Marseille, France.

A matching-to-sample task was used to assess the ability of baboons (n=8) and humans (n=14) to process the global and local information of compound visual stimuli. During the test, a compound sample stimulus, such as a large square made of smaller circles, was displayed for 120 ms in the left (LVF) or right (RVF) visual half-field. Sample presentation was followed by the display of two comparison forms, one matching the sample at either the global or local level, the other being neutral. By manipulating a joystick, the subjects had then to select the comparison form matching the sample. The two species exhibited a LVF (right hemisphere) advantage for global matching, and a reversed but unsignificant RVF (left hemisphere) advantage for local matching. In humans, scores were better and speed faster, when matching had to be made at the global level. In contrast, baboons showed a significant score and speed advantage for local matching. This species difference was replicated in 2 additional experiments, in which effects of stimulus element sparsity and subject familiarization with the forms were assessed. Overall, results suggest (1) that global dominance is not mandatory in nonhuman species, and (2) that this effect in humans has some strong cognitive, rather than purely perceptual (peripheral) bases.

820.6

CONSTRUCTION OF THE SPATIAL COGNITIVE MAP DOES NOT REQUIRE ACTIVE EXPLORATION OF THE ENVIRONMENT. D.B Matthews*, A.M. White, E.D. Brush & P.J. Best. Center for Neuroscience Research and Dept. of Psychology, Miami University, Oxford OH 45056.

When navigating, rats use distal, spatial cues to construct a spatial cognitive map (O'Keefe & Nadel, 1978). This map facilitates navigation and is apparently constructed even if the animal has never physically navigated through the entire environment. Recently, this idea has been challenged by Sutherland et al. (1987) who report that rats trained in the Morris Water maze with barriers restricting their exploration were not able to escape from a novel start location that was located within the restricted region. However, their study included severe alterations in the environment on the test day that may have impaired performance merely by a stimulus generalization decrement. The current study shows that when the rats are trained in the water maze with barriers are that are faded after the animal learns the task, escape performance is not impaired when the rats are started from a novel start location. However, when the barriers were not faded during training and were removed during testing (a method similar to Sutherland's), the animals performance was impaired. Hence, the spatial cognitive map can be used to support spatial navigation regardless of the level of exploration.

820.7

DOES THE LEVEL OF BRAIN ACTIVITY REFLECT THE PERFORMANCE OBTAINED IN TEMPORAL TASKS? L. Casini, F. Macar and O. Pascalis*. Lab. de Neurosciences cognitives, CNRS, 13420 Marseille cedex 20, France.

Attentional models of time perception assume that temporal judgments are based on mechanisms that depend on attentional resources. In order to better understand these mechanisms, slow brain potentials were recorded during different temporal tasks. The relative level of activity asssociated with correct responses was compared to that obtained with incorrect responses to study the relation between performance and brain activity. Slow brain potentials were recorded over 16 electrodes placed over the prefrontal areas.

In the first experiment subjects were tested on a temporal reproduction task: Four As appeared on a video screen for 3 or 4 sec and were then replaced by four Bs. Subjects had to press a button when they thought that the Bs had remained on the screen as long as the As. The B periods were grouped into different categories depending on their duration and three categories were compared: Accurate responses centered on the target, short responses (underestimation of 1 sec) and long responses (overestimation of 1 sec). In the second experiment, four As were presented on a video screen either with a short (2.5 sec) or a long (3.5) duration and subjects had to decide if the presented duration was the short or the long one. Correct and incorrect responses were compared.

In both experiments, correct responses were correlated with a significantly lower level of activity suggesting that efficient temporal processing is related to reduced prefrontal activation. This relation between the subject's performance and the level of brain activity suggests that the errors correspond to increased "cortival noise".

820.8

NEURAL SYSTEMS IN TEMPORAL INFORMATION PROCESSING: THE BASAL GANGLIA AND THE CEREBRAL HEMISPHERES **D.L. Harrington, K.Y. Haaland, and W.D. Stofer* Veterans Affairs Medical Center and University of New Mexico, Albuquerque, NM 87108**

Controversy exists about whether timing is localized (i.e., cerebellum) or is a distributed process regulated by many neural systems. The present study examined the role of the cerebral hemispheres and the basal ganglia in timing, as these neural structures have been linked to timing functions. Twenty right-hemisphere (RH) and 20 left-hemisphere (LH) stroke subjects, 30 subjects with Parkinson's disease (PD), and 43 control subjects were studied using procedures of Keele and colleagues (1985; 1989) for isolating timing operations. Subjects completed two motor timing tasks (i.e., a constant 300 or 600 ms intertap interval [ITI]), two perceptual timing tasks (i.e., a 300 and 600 ms SOA between tone pairs), and an auditory processing control task (i.e., frequency perception). The total variability in motor timing was partitioned into clock and motor implementation sources of variance using the methods of Wing and Kristofferson (1973). The PD group was impaired in the clock, but not the motor source of variance in the motor timing task, regardless of the ITI. Only the clock component of motor timing was impaired in the stroke groups, but a hemispheric asymmetry was found such that the RH group showed the greatest clock variability at the 600 ITI and the LH group showed the greatest clock variability at the 300 ITI. Perceptual timing was impaired in all patient groups. Only the two stroke groups were impaired on the frequency perception control task. The results implicate the basal ganglia and/or the thalamocortical circuits in timing. In addition, the findings are consistent with models that propose hemispheric biases for processing frequency information, and this asymmetry is further explored in analyses of intrahemispheric lesion location.

820.9

A PREDICTIVE MODEL FOR DIFFUSE SYSTEMS MATCHES HUMAN CHOICES IN A SIMPLE DECISION-MAKING TASK. D.M. Egelman, C. Person, P.R. Montague*. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Recent physiological work in alert primates suggests that firing rate changes in a subset of mesencephalic dopamine neurons carries information about expectations of future rewarding events. These neurons also have access to the precise time at which a reward will be delivered. We have previously proposed that the output of these neurons represents ongoing errors between the expected amount of reward and amount delivered, shown how such errors could be constructed in a real brain, and related the resulting algorithm to methods of optimal control (Quartz *et al.*, Soc. Neuro. Ab. 18:1210 (1992)). We have now used the model to bias action choice through control of noise sensitivity of target neurons. Both humans (n=9) and networks were given a simple two deck card choice task where the amount of reward from both decks was not stationary and depended on the history of choices from the decks. The networks converged quickly so as to match the relative rate of return from the two decks. The majority of humans (n=7) settled into the same 'matching' strategy while a minority performed near the optimum (n=2). Our proposed model provides one possible bottom-up description of how diffuse systems could establish constraints that favor event matching while not excluding other more complicated reward-seeking strategies. This may explain why it is difficult for animals, however rational they may be, to maximize long-term rewards and why under appropriate circumstances they display risk averse behavior.

820.10

A THEORY OF PERCEPT PROCESSING L.D. Partridge* L.D. Partridge Dept of Biomed. Eng. Univ. of Memphis, Memphis TN 38152 and Dept. of Physiol. Univ.of New Mexico, Albuquerque NM 87131

Any cognitive theory that attempts to explain sensory percepts must deal with the integration of information across sensory modalities. The ability to parse the sensory world into discrete identifiable objects is a very individual process that relies on both genetic predisposition and neuronal plasticity. As such it forms the basis for the individual's unique cognitive interaction with the world.

A useful description of processing of sensory information utilizes the concept of specific percept attractors. The set of all inputs that can activate a given attractor constitutes the basin of that attractor. Adjacent basins are separated at sepatrixes. A percept attractor is a characteristic and multidimensional neuronal path that can be loosely linked to other percept attractors in either a mutually inhibitory or nested manner. It is proposed that there is a range of attractors, from those that can be fully activated by an input of a single dimension in one sensory modality, to those that are activated by varying inputs from multiple dimensions over several sensory modalities. Individual inputs to these attractors can merely bias neurons toward a state in which an activity trajectory is sustained transiently.

We present here a theory of orders of progressively more complex sensory basins approximately representing the drive of fixed action patterns and of simple reflexes, and simple perceptions and an integrated perceptual space. The from-bottom-up meta control of sepatrix fields in the integrated perceptual space may in turn be the basis for primary consciousness.

820.11

SOLVING THE PUZZLES OF HUMAN PERCEPTION.
Y. Shapiro*. TBD. Cambridge, MA 02138.

The puzzles of perception that emerge from the computational models, which are based on Helmholtz's theory of perception, include: How must the brain compute 2D retinal images into 3D representations? Given the foveal blind spot, how must the brain compute so that we see the objects without the hole/s corresponding to the blind spot? Given the blood vessels in the retina, how must the brain compute so that we see the objects whose surfaces are not laced with the lines corresponding to the vessels? While more puzzles are certainly in the coming from the students of computer vision, the puzzle of puzzles - "fusion" has another problem yet. Purves & White (1994) tested the hypothesis of and present experimental evidence for monocular habit in normal distance viewing. Their finding is well explained by the well known fact: most neurons of primary visual cortex have preferential response to the stimulation of one eye or the other, but it contradicts the explanation of perception as "fusion". As Purves & White (1994) point out, the explanation is suspect anyway: it cannot explain either "double vision" or ocular rivalry, and it is at odds with the preferential response. "Fusion" or "the inner eye" is invoked to explain the unity of perception which begins in two retinal images of the brain that must compute retinal images. And to compute the brain must store, as it must in the new biological models of Calvin (1990) and Edelman (1989, 1992), according to which retinal images are superfluous. The assumption that neurons compute and store requires two classes of neurons for which we have no morphological, chemical, or other evidence. LTP provides no convincing evidence for LTM (Shapiro, 1994). To solve all the puzzles, a new theory of perception is badly needed and will be presented.

MONOAMINES AND BEHAVIOR: DOPAMINE II

821.1

6-OHDA LESIONS OF MEDIAL PREFRONTAL CORTEX POTENTIATE STRESS-INDUCED ANALGESIA IN THE RAT. C.A. Sorenson* and J.L. Bloom. Neuroscience Program, Amherst College, Amherst, MA 01002.

Previous research has shown that 6-OHDA lesions of medial prefrontal cortex (MPFC) produces hyperdopaminergia in the mesolimbic DA system characterized by elevated D2 receptor density, an increase in the stress-related release of DA, and an increase in behaviors linked to mesolimbic activity. Evidence also suggests a role for mesolimbic DA neurons in the production of analgesia. This study was undertaken to test whether animals receiving MPFC injections of 6-OHDA would show potentiation of stress-induced analgesia, as measured by an increase in tail flick latency (TFL) scores to mild stressors (.2 ma footshock or 60 sec. of an audiotape of rat pain-induced vocalization). Twenty-four male Sprague-Dawley rats received bilateral MPFC injections of either 6-OHDA or the ascorbate vehicle. 6-OHDA animals displayed a significantly greater increase in TFL scores following exposure to either stressor than did controls. In addition, the locomotor response to amphetamine was significantly greater in 6-OHDA animals, and there was a highly reliable correlation between locomotor activity score and the increase in TFL produced by vocalization. These findings suggest that mesolimbic DA neurons may modulate aspects of the stress response, such as analgesia, and therefore implicate these neurons in the control of emotional reactivity.

821.2

CHRONIC IN VIVO MONITORING OF EXTRACELLULAR DOPAMINE USING ELECTROCHEMISTRY. G. C. Parker* and P. B. S. Clarke. Pharmacology & Therapeutics, McGill Univ., Montreal, PQ, Canada H3G 1Y6.

We have developed a technique to monitor the changes in interstitial dopamine (DA) with a high temporal frequency in a preparation that provides consistent measurements over months rather than days. Nafion-coated carbon paste 'working' electrodes were tested after chronic implantation in the rat nucleus accumbens. In a typical session, voltammetry is used to characterise the voltage-current relationship for that implant and determine the parameters to be used in chronoamperometry. By comparing the voltammogram to the measured current at various potentials using chronoamperometry, we have demonstrated that at the sweep speed used, the voltammogram correctly describes the optimal potential to use in the chronoamperometric experiments. A session can last several hours with a stable baseline, sampling as often as every 3 sec on the device we use (GMA Echempro). Electrochemical selectivity was tested as follows. The monoamine oxidase inhibitor pargyline (100 mg/kg IP) increased the signal, whereas the D1/D2 agonist pergolide (0.6 mg/kg IP) decreased it. Uncoated electrodes monitored opposite changes from a higher baseline. The 5-HT uptake inhibitor fluoxetine (10 mg/kg IP) did not alter the signal at Nafion-coated electrodes. By reference to literature microdialysis reports, our results are consistent with the Nafion-coated electrodes measuring DA with minimal interference from DOPAC, ascorbate, 5-HT or 5-HIAA at the parameters used. Using a modification of a reference electrode design (Kruk et al., Soc Neurosci Abstr 19: 384.14) our preparation is still stable and selective more than two months after surgery. This technique provides a valuable tool for behavioural and pharmacological analyses requiring a chronic preparation, sampling with a temporal resolution higher than is commonly available using microdialysis.

821.3

HETEROGENEITY OF PRESUMED DOPAMINE NEURONS IN THE VENTRAL TEGMENTAL AREA IN AWAKE, UNRESTRAINED RATS. E. A. Kiyatkin* and G. V. Rebec, Prog. in Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405.

Mesocorticolimbic dopamine (DA) neurons play a pivotal role in the regulation of behavioral processes. Although much is known about these cells in anesthetized and in vitro preparations, information on the mechanisms regulating their activity under natural conditions is very limited. To assess this issue, single-unit recording combined with microiontophoresis was used in awake, unrestrained rats to study the electrophysiological properties of neurons in the ventral tegmental area (VTA) and their responses to DA and glutamate (Glu). In contrast to the traditional differentiation of VTA units into two groups -- DA and non-DA cells (long vs. short spike and low vs. high rate of activity) -- we found substantial diversity in both spontaneous impulse activity and responsivity to the applied compounds. Although an analysis of our data revealed at least five types of VTA neurons, two of these appeared to be DA-containing. Type I cells, localized in lateral and deep areas of the VTA, had relatively long bi- or triphasic spikes, low rate of nonbursting spontaneous activity with minimal changes after presentation of simple activating stimuli and/or during movement. These cells were highly sensitive to DA, but the threshold for the Glu response was high, and the magnitude of Glu-induced activation was small with profound rebound inhibition. Type II cells, localized throughout the dorso-medial areas of VTA, had a triphasic spike of shorter duration and discharged with a bursting pattern at a highly variable rate. These cells typically show powerful activation characterized by bursting, decreasing spike magnitude and, on occasion, episodes of depolarization inactivation, after stimuli presentation and during spontaneous motor behavior. Type II cells were highly sensitive to Glu-induced activation and consequent depolarization block, but the threshold for the DA-induced inhibition was much higher than in Type I cells. Although the reasons and mechanisms underlying this heterogeneity are unknown, VTA DA cells of both types appear to be governed by strict self-regulatory mechanisms minimizing their response to direct afferent stimulation. Supported by NIDA (DA 02451).

821.4

EFFECTS OF HALOPERIDOL AND SCH 23390 ON JUVENILE RAT SOCIAL BEHAVIOR: IMPLICATIONS FOR THE ROLE OF DOPAMINE. S. L. Bucher-Yiannoutsos*, S. Kilroy, L. Lassow, & J. D. Salamone Department of Psychology, University of Connecticut, Storrs, CT., 06269-1020

The effects of various doses of haloperidol (HP; 0.025 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.2 mg/kg, vehicle) and SCH 23390 (SCH; same doses) on juvenile rat social behavior were assessed. In two experiments, the effects of systemic drug administration on rough-and-tumble play (RT play) vs. non-playful social (NP social) activity were investigated via a modified paired-encounters paradigm. Two hundred dyads (n = 100 dyads per experiment) were tested according to dose of HP or SCH administered, and whether one or both members of the pair (1MEM or 2MEM) received an injection. Subjects were observed for 15 rather than 5 minutes, and the frequency of NP social activities (e.g., sniffing) was evaluated. Results indicate that the modified paradigm is useful in characterizing the complex nature of juvenile social behavior, as well as delineating the behavioral effects of pharmacological compounds. Haloperidol selectively affected RT play for the 2MEM sample in a dose-dependent fashion, with the 0.05 mg/kg dose resulting in excitation, and the 0.2 mg/kg dose inducing significant behavioral suppression. By contrast, HP did not significantly affect either the 1MEM sample or NP social activity at any dose administered. SCH also induced a dose-dependent decrease in RT play, and had no effect on NP social activity. Unlike HP, lower doses of SCH did not result in higher levels of playful behavior. Taken together, these results indicate that brain dopamine may be critical for the execution of RT play, but appears to be less critical for the expression of NP social behavior.

821.5

SOCIAL REACTIVITY IN INBRED MOUSE STRAINS: MEDIATION OF FEAR BEHAVIOR BY D_1 AND D_2 DOPAMINE RECEPTORS

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In previous experiments we observed that dihydrexidine (DHX), a full efficacy dopamine agonist with a 10 fold selectivity for D_1 vs. D_2 sites, markedly increased social reactivity in isolated ICR mice. This response was correlated with an isolation-induced increase in striatal D_1 density. In this study we hypothesized that these effects would be particularly robust in inbred mouse strains characterized as high in emotionality. Given the importance of motor activity in the expression of emotional behavior, two inbred mouse strains (A/J and C57BL/6J) high in emotionality and low and high, respectively, in motor activity were chosen. Isolated mice were administered either a D_1 (DHX, 10 mg/kg) or D_2 (quinpirole, 1.0-3.0 mg/kg) agonist and their behavior was assessed in a social interaction test. Both agonists induced a marked strain dependent social reactivity. In C57BL/6J mice, DHX significantly increased escape behavior whereas quinpirole (3.0 mg/kg) had only marginal effects on this measure and other measures of social reactivity. Conversely, quinpirole induced a striking increase in almost all measures of social reactivity (e.g., defensive kicking, jump, vocalization) in A/J mice. This heightened reactivity was also observed following DHX, albeit to a much lesser degree. These findings highlight the importance of dopamine receptors in the expression of fear-related behaviors. They also suggest differences in receptor subtype mediation of social reactivity as a function of strain. Supported by NSERC of Canada and MH45371.

821.6

ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERIZATION OF SINGLE NEURONS IN THE VENTRAL TEGMENTAL AREA OF THE RAT.

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The ventral tegmental area (VTA) is a central element in a neural system that mediates the reinforcing properties of natural stimuli (such as food), brain stimulation and drugs of abuse. While VTA dopaminergic neurons appear to be of particular importance, the VTA also contains interneurons and non-dopaminergic projecting neurons whose role in VTA function and influence on dopamine release are relatively unknown. Using multiple chronically implanted microwire electrodes, it is possible to record from many VTA neurons simultaneously. In preparation for studies of the functional interaction of VTA neurons, we have been developing criteria for identification and subgrouping of recorded neurons. In the present studies, bundles of 4-8 microwire electrodes were chronically implanted in the VTA of male Wistar rats. Following recovery from surgery, recordings were obtained in awake and anesthetized rats during administration of drugs including apomorphine (a mixed D1/D2 agonist), morphine or alfentanyl (μ opiate agonists), and midazolam or diazepam ($GABA_A$ agonists). Signals reflecting the activity of single neurons were identified by uniformity of waveform, and decreasing probability of unit firing observed around the node of the autocorrelation histogram. Neurons broadly classifiable as dopaminergic (long duration, irregularly shaped waveforms, relatively slow firing rates, inhibited by apomorphine, excited by μ opiate and $GABA_A$ agonists) and non-dopaminergic (short duration, biphasic action potentials, excited by apomorphine, inhibited by μ opiate and $GABA_A$ agonists). Furthermore, the heterogeneity observed within these two broad categories suggests that it may be possible to further subdivide these neurons, for instance into non-DA interneurons and projecting neurons, and also into subtypes of DA neurons. Supported by NIDA DA 08349 and Hahnemann University RIG 990414.

821.7

DOSE-DEPENDENT EFFECTS OF ASCORBATE ON CONDITIONED AVOIDANCE RESPONSE. J. M. Gulley* and G. V. Rebec, Program in Neural Science and Dept. of Psychology, Indiana University, Bloomington, IN 47405.

Pretreatment with ascorbate, a known modulator of dopamine transmission in the striatum, enhances the ability of haloperidol, a dopamine antagonist, to induce catalepsy and to block the behavioral effects of amphetamine (Rebec and Pierce, Prog. Neurobiol., 43:537, 1994). In the present study, we extended this line of work to the lever-release version of the conditioned avoidance response (CAR) task, which is highly sensitive to changes in striatal dopamine (White and Rebec, Neurosci. Protocols, in press). Increases in dopamine transmission enhance CAR performance, while decreases have the opposite effect. Adult, male rats were trained to avoid footshock by releasing a lever within 500 msec of tone onset. We tested the effects of low (100 mg/kg) and high (1000 mg/kg) dose ascorbate both alone and in combination with haloperidol (.01 and .05 mg/kg). Consistent with previous results, high dose ascorbate alone impaired CAR performance and increased response latency without altering the latency of the escape response. The low dose of ascorbate, in contrast, failed to impair CAR performance by itself and attenuated the detrimental effects of haloperidol. Collectively, these results confirm and extend reports that ascorbate modulates dopamine-mediated behavioral responses. Our data suggest, however, that this effect of ascorbate is dose-dependent such that low doses enhance and high doses impair striatal dopaminergic mechanisms.

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821.8

EFFECTS OF L-DOPA, METHAMPHETAMINE AND APOMORPHINE ON SHUTTLE AVOIDANCE LEARNING IN NEONATALLY 6-OHDA-TREATED RATS. M. Takasuna*[1], T. Okauchi[2] and T. Iwasaki[2]. [1] Yamano College of Aesthetics, Tokyo 192-03, Japan. [2] Institute of Psychology, Univ. of Tsukuba, Tsukuba 305, Japan.

Neonatal depletion of brain dopamine produces various behavioral deficits including hyperactivity as well as learning impairment of active avoidance task (Takasuna et al, 1995). To investigate the drug effects on shuttle avoidance learning in the dopamine-depleted rats, L-DOPA (5 or 25 mg/kg, i.p., pretreated with benserazide), methamphetamine (1 or 2 mg/kg, i.p.) and apomorphine (0.5, 1.0 or 10 mg/kg, i.p.) were injected before the training session. Rats were treated with 6-hydroxydopamine (6-OHDA; 35 μg/3 μl/ventricle, pretreated with desipramine) on Days 2 and 4 after birth. The avoidance training (50 trials x 3 days) began on Day 90 after 5-min open-field test. Though each drug elicited a dose-dependent alteration of activity in the open-field, none of the drugs increased the number of avoidance responses in 6-OHDA-treated group. In particular, the increase of intertrial shuttling responses after the injection of higher dose of L-DOPA was not accompanied with improvement of avoidance performance. These results suggested that the performance of 6-OHDA-treated rats was not affected by the activity-stimulating effect of the drugs.

821.9

EXPOSURE TO AMPHETAMINE *IN VIVO* LEADS TO ENHANCED AMPHETAMINE-INDUCED DOPAMINE RELEASE BY DOPAMINE NEURON TERMINALS *IN VITRO*. M. O'Neill, J. Wang and P. Vezina*. Department of Psychiatry, University of Chicago, Chicago, IL 60637.

Experiments investigated the effect of prior exposure to systemic injections of amphetamine on the subsequent release of dopamine (DA) evoked by amphetamine from DA neuron terminals in the rat nucleus accumbens (N.Acc.) and dorso-lateral striatum (Dl.Str.) *in vitro*. Different groups of rats were administered five injections of amphetamine (3.0 mg/kg, i.p.) or saline, one injection every third day. Two weeks following the last injection, rats were decapitated, their brains rapidly removed, the N.Acc. and Dl.Str. bilaterally dissected out and the tissue incubated in oxygenated sodium bicarbonate buffer. Tissue was incubated repeatedly for five minutes in 400 μl of medium and exposed in successive incubations to different concentrations of amphetamine (0-10 μM). Prior exposure to amphetamine *in vivo* led to significantly enhanced, concentration dependent, amphetamine-induced DA release from both N.Acc. and Dl.Str. DA neuron terminal tissue *in vitro*. The two groups did not differ when no amphetamine was present in the medium: basal DA release *in vitro* was not altered by amphetamine preexposure *in vivo*. These results confirm and extend those of previous reports (Kolta *et al.*, Neuropharmacol., 1985, 24, 823; Castaneda *et al.*, Life Sci., 1988, 42, 2447). Together with other findings indicating that amphetamine acts in the cell body region of midbrain DA neurons to produce sensitization, these results support the view that exposure to such injections leads to long term changes in midbrain DA neuron function.

821.10

MK-801 BLOCKS THE DEVELOPMENT OF SENSITIZATION TO THE LOCOMOTOR AND DOPAMINE ACTIVATING EFFECTS OF AMPHETAMINE. D.C. Jolly* and P. Vezina. Department of Psychiatry, University of Chicago, Chicago, Illinois 60637.

Repeated intermittent administration of amphetamine has been shown to produce an enhanced locomotor and nucleus accumbens (N.Acc.) dopamine (DA) response to a subsequent challenge injection of amphetamine, when testing is done sufficiently long after the last drug injection. This study used *in vivo* microdialysis and measured locomotor activity in rats to examine whether these effects would develop in the presence of NMDA receptor blockade by MK-801. Four groups of rats were tested. During preexposure, rats in each group received 2 injections (separated by 30 min.) per session. These were conducted every third day. Rats in the S-S group received two injections of saline. Rats in the S-A group each received saline and amphetamine (1.0 mg/kg, i.p.). Rats in the M-S group received MK-801 (0.3 mg/kg, s.c.) and saline. Finally, rats in the M-A group received MK-801 and amphetamine. This procedure was repeated five times. Two to three weeks after the last injection, all animals were given amphetamine (1.0 mg/kg, i.p.) and their locomotor and N.Acc. DA response measured. The S-A rats showed a significantly greater locomotor and N.Acc. DA response in comparison to S-S rats, reflecting sensitized responding by S-A animals. In contrast, the locomotor and N.Acc. DA response of M-A rats did not differ significantly from those of M-S. rats. These results suggest that NMDA receptor activation is a necessary component of the development of sensitization to amphetamine. Interestingly, both the locomotor and N.Acc. DA responses of animals having been preexposed to MK-801 were higher than those of S-S rats. This may reflect an additional and separate long-term effect of exposure to this NMDA receptor antagonist.

821.11

CROSS-SENSITIZATION IN THE PREWEANLING RAT: THE EFFECTS OF DIZOCILPINE ON AMPHETAMINE AND NPA. M.A. Duke, C.A. Bolanos, G.M. Garmsen, M.A. Clair, S.A. McDougall*. Dept. of Psychology, California State Univ., San Bernardino.

Repeated exposure to a variety of direct (NPA) or indirect (amphetamine; AMPH) DA agonists produces sensitization in young and adult rats. NMDA antagonists (dizocilpine; DIZ) block the induction of sensitization in adults, suggesting that DIZ and the DA agonists AMPH and NPA share a common locus of action. To further assess this possibility, we attempted to determine, using the preweanling rat, whether (1) DIZ would block the sensitization induced by AMPH and NPA; and (2) whether DIZ, AMPH, and NPA are capable of inducing cross-sensitization. 17-day-old rats were injected on four consecutive days with DIZ (0.3 mg/kg) or saline. After 30 min, rats were then given AMPH (2.5 mg/kg), NPA (1.0 mg/kg), DIZ, or saline. A final test day occurred 2 days later, with activity, sniffing, and rearing being measured after AMPH, NPA, or DIZ treatment. The results showed that pretreatment with DIZ blocked the sensitization induced by AMPH and NPA. Curiously, DIZ, AMPH, and NPA did not induce cross-sensitization in the 17-day-olds. For example, repeated treatment with AMPH had no effect on the behavioral actions of DIZ or NPA. Thus, these results show that DIZ, AMPH, and NPA are each capable of inducing sensitization, but that the sensitization produced by these drugs may be mediated by different mechanisms.

821.12

CUES SPECIFICALLY UNPAIRED WITH AMPHETAMINE ATTENUATE THE DEVELOPMENT OF LOCOMOTOR SENSITIZATION TO THE D-2 DOPAMINE RECEPTOR AGONIST QUINPIROLE. C. Jake-Matthews* and P. Vezina. Department of Psychiatry, University of Chicago, Chicago, IL 60637.

Experiments were conducted to determine whether prior exposure to amphetamine enhances the subsequent development of sensitization to the locomotor effects of the D-2 dopamine receptor agonist quinpirole. Because conditioned drug effects have been shown to influence the expression of sensitization to amphetamine, the role of such effects in the development of sensitization to quinpirole were also assessed.

Three groups of rats were first administered injections of either amphetamine (1.5 mg/kg, i.p.) or saline. Animals in one group (PAIRED) received amphetamine in locomotor activity measuring boxes and, the following day, saline in their home cage. Animals in a second group (UNPAIRED) received saline in the activity boxes and amphetamine in their home cage. Animals in a third group (CONTROL) received saline in both environments. All animals were left undisturbed on the third day. This procedure was repeated five times. Seven to ten days following the last injection, all animals were administered quinpirole (0.5 mg/kg, s.c.) in the activity boxes and their locomotor response measured. These injections were repeated every four days for a total of 10 quinpirole injections.

Prior exposure to amphetamine had no effect on the subsequent development of sensitization to the locomotor effects of quinpirole. Both PAIRED and CONTROL group animals displayed a similar development of sensitization to quinpirole when this agonist was administered repeatedly. Interestingly, the development of sensitization to quinpirole over days in UNPAIRED group animals was significantly retarded relative to the other two groups. These results suggest that while different mechanisms likely contribute to the locomotor sensitization observed with amphetamine and D-2 dopamine receptor agonists, both instances are dramatically affected by conditioned stimuli specifically unpaired with drug.

821.13

LONG-TERM, BI-WEEKLY TREATMENT OF LESIONED RATS WITH QUINPIROLE LEADS TO BEHAVIORAL SENSITIZATION TO BOTH D1 AND D2 DA RECEPTOR AGONISTS AND TO L-DOPA. K.E. Asin*, L. Bednarz and A. Nikkel, Neuroscience Res. D-47U, Pharmaceutical Products Division Abbott Laboratories, D-47U, Bldg AP9A, Abbott Park, IL 60064

Reports indicate that daily, repeated administration of selective D1 and D2 dopamine (DA) receptor agonists to lesioned rats may lead to the development of behavioral tolerance or sensitization in response to an acute drug challenge. Since sensitivity changes are dependent on dose and treatment schedule, we investigated the consequences of intermittent, repeated treatment of lesioned rats with vehicle (**V**), D1 or D2 DA agonists on rotation after acute D1 and/or D2 agonist challenges.

Male Sprague-Dawley rats were given unilateral injections of 6-OHDA into the MFB. Following recovery, rats were tested for rotation after apomorphine (0.3 mg/kg), divided into 4 groups (N=17-18/grp), and given 43, bi-weekly injections of either **V**, the D1 agonist **A-85653** (0.09mg/kg), quinpirole (0.15mg/kg) (**Quin**) or A-85653 (0.03mg/kg) + Quin (0.05 mg/kg) (**COMBO**); rotation was examined periodically. After this repeated treatment phase (RTP), animals were challenged with various doses of either A-85653, Quin, or L-DOPA and rotation was measured.

During the RTP, all but **V** rats demonstrated behavioral sensitization. Repeated **A-85653** led to behavioral tolerance in response to acute, low doses of A-85653, but response sensitization following a higher dose compared to rats given **V** during the RTP. Rotation levels were unchanged compared to **V** following any challenge dose of Quin, but the behavioral response to L-DOPA was diminished. In contrast, rats given **Quin** during the RTP showed enhanced rotation in response to all compounds, including A-85653. The effects of repeated **COMBO** treatment were compound- and dose -dependent and tended to reflect the additive effects of repeated A-85653 and Quin. HPLC analysis indicated that rats sustained 95% striatal DA depletions.

Our results indicate that repeated quinpirole treatment leads to behavioral supersensitivity in rats following an acute injection with a D1 or D2 receptor agonist or L-DOPA. In contrast, repeated A-85653 treatment produces dose-dependent changes in behavioral sensitivity to itself, but not to quinpirole or L-DOPA.

821.14

CORRELATION OF STRIATAL DYNORPHIN mRNA EXPRESSION AND BEHAVIORAL SENSITIZATION FOLLOWING REPEATED D1 AGONIST INJECTION. J.F. Bejar, M.B. Harrison, and J.M. Trugman* Department of Neurology, Univ. of Virginia, Charlottesville, VA 22908

Repeated injections of D1 dopamine agonists have been shown both to elicit behavioral sensitization and increase striatal dynorphin mRNA expression but the correlation between dynorphin expression and sensitization has not been established. Rats with unilateral 6-hydroxydopamine nigral lesions received 1, 2, 4, or 8 daily injections of the D1 agonist SKF 38393 (10 mg/kg ip). Contralateral rotation was observed for 2 hours following each injection and after the last injection the brains were processed for dynorphin mRNA expression by *in situ* hybridization. Rotation increased as a function of the number of injections with a maximal response achieved after 5 injections (repeated measures ANOVA, $P<0.05$). Dynorphin mRNA expression was markedly increased in the dopamine deafferented striatum after 4 and 8 injections of D1 agonist with the most prominent effect seen in the dorsolateral quadrant (up 250%). Dynorphin expression and rotation both positively correlated with injection number (linear regression analysis, $P<0.05$) and there was a positive association of dynorphin expression and rotational response (Spearman rank-order correlation, $P<0.05$). These results are consistent with the hypothesis that increased striatal dynorphin contributes to the enhanced motor response resulting from repeated D1 agonist injection.

821.15

STRIATAL DOPAMINE CONCENTRATIONS FOLLOWING AN ACUTE TREATMENT WITH AMPHETAMINE IN A NOVEL VERSUS HOME ENVIRONMENT. K. E. Browman *, A. Badiani, J. J. Lalley and T. E. Robinson. Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI 48104.

The magnitude of amphetamine-induced rotational behavior is greater in rats treated in a NOVEL versus HOME environment (Badiani et al. 1993). We hypothesized that the environment may influence the behavioral response to amphetamine by modulating striatal dopamine concentrations. In a previous studies, however, we found no HOME versus NOVEL differences in the effects of amphetamine on dopamine concentration in the NAcc-Core. These experiments were to determine whether HOME versus NOVEL differences in amphetamine-stimulated dopamine may be found in the NAcc-Shell or in the Caudate.

Rotational behavior in rats with a unilateral 6-OHDA lesion of the mesostriatal dopamine system was used as an index of the psychomotor activating effects of amphetamine. The animals were implanted contralaterally with a microdialysis probe aimed at either i) the NAcc or the Caudate (Experiment 1), or ii) the NAcc-Shell (Experiment 2). One day later the animals received 2 mg/kg of amphetamine IP, either in their HOME cage or in a NOVEL test cage. Rotational behavior was quantified for 100 min. and simultaneously samples of dialysate were collected every 20 min. In Experiment 1 we found that, although amphetamine-induced rotational behavior was enhanced in the NOVEL environment, there were no differences in NAcc or Caudate dopamine. Experiment 2 is in progress, and data will be reported at the meeting.

821.16

THE DEVELOPMENT AND EXPRESSION OF AMPHETAMINE SENSITIZATION ARE ATTENUATED BY REMOVING TREATMENT ASSOCIATED CUES. Hans S. Crombag*, Aldo Badiani and Terry E. Robinson. Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI 48104.

The development and expression of sensitization to the psychomotor stimulant effects of amphetamine (AMPH) is significantly attenuated if rats are given drug treatments in their HOME cage relative to rats receiving the same treatments in a physically identical but NOVEL test cage (Badiani, 1993). One difference between the HOME and NOVEL environment is the salience of cues that predict drug administration. We hypothesized, therefore, that removal of all cues predictive of drug administration would further attenuate the development and expression of sensitization. We tested this hypothesis by comparing AMPH sensitization in two groups of rats. One group received an unsignalled intravenous (i.v.) infusion of AMPH (1.0 mg/kg) each day for six consecutive days at HOME using a remotely activated infusion pump. The second group received the same treatment but in a NOVEL environment. The psychomotor response to AMPH was quantified by measuring rotational behavior in unilaterally DA-denervated rats. Repeated AMPH treatment produced a progressive increase in drug response in rats that received i.v. infusions in the NOVEL environment. In contrast, the same dose of AMPH produced a very small acute response in the HOME environment and there was no evidence of sensitization. These results further implicate the role of environmental factors in the development and expression of behavioral sensitization.

821.17

FURTHER STUDIES ON THE ENHANCING EFFECTS OF A NOVEL VERSUS HOME ENVIRONMENT ON AMPHETAMINE SENSITIZATION. A. Badiani* and T. E. Robinson. Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI 48104.

Sensitization to the psychomotor activating effects of amphetamine is greater in rats treated in a NOVEL versus HOME environment (Badiani et al. 1993), as indicated by a greater rate of sensitization (i.e., the progressive increase in amphetamine effects over consecutive test sessions). In the present study we report on NOVEL versus HOME differences in the effects of intermittent amphetamine on two other indices of amphetamine sensitization.

Rotational behavior in rats with a unilateral 6-OHDA lesion of the mesostriatal dopamine system was used as an index of the psychomotor activating effects of amphetamine. In Experiment 1 HOME and NOVEL rats received seven consecutive IP injections of either saline or 2 mg/kg amphetamine. Following one week withdrawal the rotational response to 2 mg/kg amphetamine IP was compared in saline versus amphetamine pretreated animals. Although both HOME and NOVEL groups did sensitize, the magnitude of sensitization was greater in NOVEL rats. In Experiment 2 a dose-effect curve (0.75, 1.5, 3.0, and 6.0 mg/kg amphetamine IP) was quantified before and after six IP injections of 4.0 mg/kg amphetamine. Sensitization was indicated by a parallel shift to the left of the dose-effect curve in both groups, but this shift was about 3.5 times greater in the NOVEL group than in the HOME group.

821.18

CHANGES IN DIALYSIS MEASURES OF STRIATAL MONOAMINES AND AMPHETAMINE-EVOKED ROTATIONAL BEHAVIOR AFTER ELECTROLYTIC LESION OF THE NIGROSTRIATAL BUNDLE. E. Castañeda*, I.Q. Whishaw, M. Davidson, L. Schuster, T. Daugherty and Y.-H. Li. Arizona State University, Tempe, AZ 85287-1104.

Adult male Long-Evans rats were tested for basal levels of dopamine (DA), its major metabolites and 5-hydroxyindoleacetic acid (5-HIAA) using removeable microdialysis probes implanted bilaterally into the caudate nuclei. Following baseline measures, an electrolytic lesion was created in the nigrostriatal bundle within the lateral hypothalamus using a previously implanted monopolar electrode (2 mA, 20 sec) and testing continued for 3 hr. The next day, rats received an injection of d-amphetamine (AMPH, 1.5 mg/kg, s.c.) during dialysis testing. Afterwards, the probes were removed and animals were returned to their home cages. On day 14 post-lesion, rats were tested again for basal and AMPH-evoked changes in extracellular monoamine levels. Quarter turns were measured throughout testing. Immediately after the lesion, DA levels declined in the damaged hemisphere and this was accompanied by transient rotational behavior ipsilateral to the lesion. The next day, basal DA levels remained low in the damaged side but AMPH-stimulated levels were enhanced relative to the intact side and rats turned predominantly contralateral to the damaged side. Two weeks later, the enhancement in AMPH-evoked DA was no longer observed, although animals now turned predominantly in an ipsiversive direction. There were no hemispheric differences in DA metabolite levels at any time, but 5-HIAA levels were significantly attenuated in the damaged side on day 14 post-lesion. The present results will be discussed with regard to novel insight about loss/recovery of function.

821.19

CEREBRAL HEMISPHERE-DOMINANCE INFLUENCES BEHAVIORAL RECOVERY AFTER FETAL MESENCEPHALIC GRAFTING IN THE RAT PARKINSON MODEL. C. Rosenthal[1], J. Oertel[2], A. Brandis[2], M. Samii[1], G.F. Walther[2], G. Nikkhah[1]* [1]Neurosurgical Clinic, Nordstadt Hospital, Haltenhoffstr. 41, D-30617 Hannover [2]Institute of Neuropathology, Hannover Medical School, Konstanty-Gutschow-Str.8, D-30625 Hannover, Germany.

The improvement of spontaneous sensorimotor functions after unilateral grafting of fetal ventral mesencephalon in Parkinson patients as well as in the respective animal-models has so far been incomplete and variable. Usually, there is no good correlation between behavioral recovery and transplant survival or graft-derived reinnervation. Therefore we have investigated, whether the extent of forepaw handedness (preference) influences the level of functional recovery in tests of skilled forelimb use and stepping behaviour. 120 female Sprague Dawley rats were divided into left-handed, right-handed and ambidextrous animals on the basis of their skilled forelimb performance. The rats received unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway either ipsi- or contralaterally to the preferred paw. Rotational and sensorimotor behaviour was assessed post lesion and 5, 18, 32, 45 and 58 weeks post grafting. Finally, the number of surviving dopaminergic neurons and graft volumes were determined. The results showed a significant differences in the extent of behavioral recovery between animals grafted into the dominant vs non-dominant hemisphere. In accordance with previous studies, morphological parameters did not seem to correlate with behavioral performance.
The findings of the present study indicate that the extent of functional recovery induced by dopaminergic grafts is, at least partly, influenced by cerebral hemisphere dominance.

821.20

INTRAVENTRICULAR INJECTION OF ANTI-DβH-SAPORIN: PRELIMINARY BEHAVIORAL FINDINGS. C. C. Wrenn*, M. J. Picklo, D. Robertson, R. G. Wiley. Dept. of Pharmacology, Vanderbilt Univ., Nashville, TN 37232.

Lesions have long been used to surmise neural function from the associated changes in behavior. Anti-neuronal immunotoxins offer an efficient way to produce highly specific lesions. The objective of the current study was to examine some behavioral effects of intraventricular (i.c.v.) injection of the anti-neuronal immunotoxin anti-DβH-saporin (α-DβH-sap). This immunotoxin (IT) consists of a monoclonal antibody to the noradrenaline synthesizing enzyme dopamine β-hydroxylase (DβH) coupled by a disulfide bond to saporin, a ribosome inactivating protein. α-DβH-sap is expected to produce selective neural lesions because DβH is an enzyme located in the membrane of synaptic vesicles and nerve terminals of noradrenergic and adrenergic neurons.. Groups of rats (n=7) received injections into the left lateral ventricle of 5μg or 10μg of α-DβH-sap. These doses were chosen based on previous experiments in which we observed that the locus coeruleus could be completely lesioned with sparing of A5 neurons at a 5μg dose while both populations are killed at 10μg. One week after surgery both groups of the IT-injected rats lost weight in a statistically significant, dose-dependent manner. By two weeks, only the 10μg group differed significantly in weight loss from controls. Approximately two weeks after surgery, tail flick behavior and exploratory activity (day and night) of the groups was examined. None of the groups differed in the latency to flick the tail in response to a noxious stimulus (heat). Night-time exploratory activity was significantly decreased in both immunolesioned groups while the decrease observed during the day did not reach statistical significance. Grooming bouts were also counted during activity monitoring and were found to increase dose dependently although statistical significance was obtained only during the day between the 10μg group and control group. We conclude that i.c.v. injections produce profound and selective destruction of CNS noradrenergic neurons resulting in subtle but significant changes in behavior. Rats treated with i.c.v. α-DβH-saporin should prove useful in a variety of studies of CNS noradrenergic neural systems. (Supported by Dept. Of Veterans Affairs).

821.21

REMOVAL OF MEDIAL TEMPORAL-LIMBIC REGIONS RESULT IN ABNORMAL DOPAMINE NEUROTRANSMISSION IN THE RHESUS MONKEY.
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We demonstrated previously that neonatal limbic lesions of the medial temporal lobe area result in abnormal dopamine neurotransmission in the caudate nucleus in adult rhesus monkeys. The present study compared the effects of neonatal limbic lesions with similar lesions made in adult monkeys on dopamine neurotransmission using *in vivo* microdialysis. Three groups of adult monkeys were used in the investigation: normal adults (N=3), monkeys who had undergone surgical removal of limbic areas including the hippocampus and entorhinal cortex in adulthood (N=2), and monkeys with similar lesions made in the first 3 weeks of their post-natal life (N=3). Extracellular dopamine overflow was measured in the caudate nucleus in response to low (20mM) and high (50mM) dose potassium (K+) challenges after gas anesthesia. Microdialysis probes were positioned into the caudate nucleus, perfused with normal CSF with samples collected and analyzed for dopamine. Each study was replicated twice in each animal with a minimum of 2 probes per study. After establishing a stable baseline, a 20mM K+ challenge was given for 25 min., followed by perfusion with normal CSF for 3 hr., followed by a 2nd K+ (50mM) challenge. Samples were collected for an additional 2 hr before termination of the study. 20mM potassium challenge resulted in 56% increase in normal adults and 37% increase in monkeys with neonatal lesions. 50mM K+ challenge resulted in 505% in normal adults and 247% increase in monkeys with neonatal lesions. In contrast, no changes in caudate dopamine overflow were observed in monkeys with adult limbic lesions following either low or high potassium challenge. These results suggest that limbic lesions in rhesus monkeys have profound effects on caudate dopamine transmission that vary in magnitude depending on age when the lesion occurred.

821.22

EFFECTS OF DISTRACTION, DELAY DURATION AND DRUG TREATMENTS ON DELAYED RESPONSE PERFORMANCE IN NORMAL MONKEYS USING AN AUTOMATED BEHAVIORAL TESTING SYSTEM.
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Normal macaque monkeys were trained to perform an automated delayed response (DR) task with or without cue (attention) or delay (memory) distractors, or with variable duration delays, ranging from 0.1 sec. to 30 sec. Cue distractors negatively affected task performance while delay distractors had no effect on performance. Distractors presented during response choice also had no effect on performance. Performance deteriorated with long duration delays. Interestingly, performance did not significantly deteriorate with short duration cue presentations. Methylphenidate further impaired DR performance with cue distractors while LY-171555 and dihydrexidine had no significant positive or negative effects on DR performance. Neither methylphenidate, LY-171555, nor dihydrexidine further affected performance of long delay DR trials. Scopolamine (0.01 mg/kg) significantly impaired DR performance but the deficit did not appear to be delay dependent. IDRA 21 (0.5 mg/kg), which prevents AMPA receptor desensitization and increases excitatory synaptic strength, had no effect on task performance alone but when given 10 min. prior to scopolamine, blocked the detrimental effects of scopolamine on DR performance. These results demonstrate the utility of our automated behavioral testing system for assessing attention, memory, and pharmacological manipulations of behavior in non-human primates.
Supported by grant MH-46531.

MONOAMINES AND BEHAVIOR: NOREPINEPHRINE

822.1

IMPACT OF PSYCHOSOCIAL STRESS ON CENTRAL ALPHA$_2$-ADRENOCEPTORS.
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Alpha$_2$-adrenoceptors are supposed to function as autoreceptors and to regulate the activity of the nor/adrenergic system in the brain. We have previously shown in male tree shrews (*Tupaia belangeri*) that under psychosocial stress (PSS), when the nor/adrenergic system is activated, binding sites for the subtype non-selective antagonist ^{3}H-rauwolscine are down-regulated. In the present study we investigated binding of the alpha$_{2A}$-subtype preferring ligand ^{3}H-RX 821002 after different durations of PSS by in vitro receptor autoradiography. Down-regulation of alpha$_{2A}$-adrenoceptors is region-specific and depends on the duration of PSS: In the locus coeruleus, the receptor number is reduced after 2, 10, and 21 days of PSS and returns to normal levels thereafter. In the prefrontal cortex, the receptor number is only slightly reduced on day 2 of PSS and is normal thereafter. In contrast, in the solitary tract nucleus and the dorsal motor nucleus of the vagus, down-regulation occurs only after 21 and 28 days of PSS. Our data demonstrate the dynamic changes in alpha$_{2A}$-adrenoceptors under PSS and indicate that in different brain regions, receptor regulation underlies different mechanisms.
(Supported by the German Science Foundation; SFB 406).

822.2

EFFECT OF ACUTE OR CHRONIC RESTRAINT STRESS ON BEHAVIOR AND BRAIN NOREPINEPHRINE SYSTEM IN WISTAR-KYOTO (WKY) RATS.
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We have reported that WKY rats develop more restraint-induced gastric ulcers and exhibit more depressive behavior than several other rat strains. Recently we have shown that repeated exposure to novel stressors for 21 days not only exacerbates depressive behavior in WKY rats but also alters ß-adrenoceptors (ß-ARs) and norepinephrine transporter (NET) sites in several limbic brain regions when compared to Sprague-Dawley rats. The present study examined whether these effects would be elaborated following an acute stress and whether WKY rats would demonstrate adaptation after repeated stress. Rats were subjected to a 2h supine restraint stress either for 1 or 8 consecutive daily sessions. Open field behavioral data were collected before the first day and immediately after the seventh session. Rats were sacrificed following their last session, brains removed, frozen and sectioned for autoradiographic analysis of ^{125}-I-pindolol binding to ß-ARs and ^{3}H-nisoxetine binding to NET sites in discrete brain regions. Acute stress resulted in a significant drop in body weight and an inhibition of behaviors in the open field. These effects were also sustained following chronic restraint stress. In contrast, while acute stress had no effect on the NE system, chronic stress decreased ß-ARs in the cortex, hippocampus and caudate putamen and decreased NET sites in the hypothalamus and locus coeruleus.
(Research funds from VA Medical Research Services and USPHS grant NS 31699).

822.3

MICROINJECTION OF NOREPINEPHRINE (NE) INTO THE MEDIAL PREOPTIC-ANTERIOR HYPOTHALAMUS (MPOA-AH) REGULATES AGONISTIC BEHAVIOR IN FEMALE SYRIAN HAMSTERS.
A.C.Harmon, K.L.Huhman, T.O.Moore* and H.E.Albers. Lab. Neuroendocrinol. & Behav., Depts. Biol. and Psychol., Georgia State Univ., Atlanta, GA 30303: Dept. Psychol. Morehouse College., Atlanta, GA 30314.

Vasopressin (AVP) within the MPOA-AH is involved in the regulation of aggressive behavior and flank marking. In females, injection of NE into the MPOA-AH inhibits the ability of AVP to induce flank marking. The present study examined whether NE injected into the MPOA-AH can alter agnostic behavior in female hamsters. Female hamsters were allowed to establish stable dominant/subordinate relationships. Dominant females were implanted with guide cannulae aimed at the MPOA-AH and injected with NE 300ng in 200nl saline or vehicle in a counterbalanced order. Following injection of NE, dominant behaviors (e.g. attacks, bites) significantly declined (Saline: 5.5±2.25; NE: 0.17±0.15; $p<0.05$) and subordinate behaviors (e.g. retreats, defensive postures) significantly increased (Saline: 0.33±0.14; NE: 4.0±1.63; $p<0.05$). No significant changes were observed in a range of other behaviors unrelated to dominance/subordinance. These data support the hypothesis that injection of NE into the MPOA-AH can regulate agonistic behavior in female hamsters.

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822.4

DEFENSIVE BEHAVIOR INCREASES MAO-A ACTIVITY IN THE LOCUS COERULEUS OF THE RAT.
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The objective of this study was to determine if monoamine oxidase (MAO) activity in rats changes as a result of an aggressive interaction. Male Sprague Dawley rats (450-500g) were allowed to establish residence in large wire cages over a period of 10 days. Naive intruders (250-275g) were introduced into the resident's cage for a period of 30 min and sacrificed immediately after the interaction. During this period, 4.75±1.03 roll-tumble fights occurred. Control rats from the same group as the intruders were sacrificed without being subjected to an intruder experience. Brain sections from the level of the locus coeruleus (LC) were assayed for MAO-A, while brain sections from the level of the dorsal raphe nucleus (DRN) were assayed for MAO-B. A histochemical, coupled peroxidatic oxidation assay utilizing nickel enhanced DAB staining was employed (Maeda et al., Cell Mol Bio., 33:1-11). The amount of DAB staining within individual cell bodies of the LC and DRN was digitized to provide a semi-quantitative measurement of MAO activity, with precise anatomical localization. MAO-A activity in the noradrenergic cell bodies of the LC was found to be 25% greater in intruders than in controls. MAO-B activity in the DRN cell bodies did not differ between intruders and controls. These results suggest the existence of a rapid mechanism for MAO-A activation in the LC which is triggered by intruder status (Supported by ASPET, Sigma Xi, and the Smithers Foundation).

822.5

DSP-4 LESIONS IMPAIR ORIENTING BEHAVIOR IN RATS.
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The orienting reflex (OR) is an early stage of sensory information processing seen as a decrease in heart rate and desynchronization of the EEG in response to a salient sensory stimulus, and may involve the locus coeruleus-norepinephrine pathway (LC-NE). For example, a role for the LC-NE in attention and vigilance has been hypothesized, and neurons in the LC-NE of awake animals respond best to salient sensory stimuli (Aston-Jones, 1985). Stimulation of the LC-NE desynchronizes EEG and slows heart rate (Berridge *et al.*, 1993; Miyawaki *et al.*, 1991).

Rats were chronically implanted for ECG and EEG recordings. The OR was elicited by taped rat vocalizations in a sound attenuating chamber. Norepinephrine was depleted by DSP-4 injections (50 mg/kg, i.p.), a selective neurotoxin for norepinephrine, and tested for an OR 11-16 days after injection.

In control animals (n=9) there was an 8.5% decrease ($p<.05$) in heart rate (HR) 4 s after the onset of the orienting stimulus (rat vocalizations), as compared to baseline HR (6 s before the orienting stimulus). HR decreased by a maximum of 10% ($p<.01$) at 13 s after the orienting stimulus and remained significantly decreased throughout the 60 s testing period, although some habituation was seen. In the DSP-4 pre-treated rats (n=8) there was a small, non-significant decrease in HR during the testing period.

Central NE concentrations in control and DSP-4 treated rats were determined by HPLC with electrochemical detection. NE concentrations were reduced in the DSP-4 animals by 31% in frontal cortex, 87% in parietal cortex, and 59% in the hippocampus.

We conclude that there is an involvement of central NE in the orienting reflex of rats.

822.6

TIME COURSE OF CHANGES IN HYPOTHALAMIC AND HIPPOCAMPAL NOREPINEPHRINE DURING AMPHETAMINE WITHDRAWAL.
D. M. Camp*, D.K. DeJonghe and T. E. Robinson. Dept. of Psychology and Neuroscience Program, Univ. of Michigan, Ann Arbor, MI 48109.

Following the discontinuation of an escalating dose amphetamine (AMP) pretreatment regimen rats exhibit a decrease in spontaneous nocturnal motor activity that persists for 1-2 weeks. Furthermore, these transient changes in behavior are associated with depletion of hypothalamic norepinephrine (NE) concentrations (Paulson et al., *Psychopharmacol.* 1991, *103*, 480-492). In the present experiment we used in vivo microdialysis to further characterize NE neurotransmission during AMP withdrawal. Animals received an escalating dose AMP pretreatment regimen (1→10 mg/kg) over 10 consecutive days and then were withdrawn for either 24 hrs or 30 days prior to determination of NE in either the hypothalamus or hippocampus during basal 'resting' conditions and also following a challenge injection of 2.0 mg/kg d-AMP i.p.. It was found that during the early withdrawal period, basal extracellular NE was significantly elevated in both brain regions, relative to saline-pretreated rats. In addition, in the hypothalamus there was a significant attenuation in AMP-stimulated NE release. Following 30 days of withdrawal, basal hypothalamic extracellular NE did not differ from controls, but there was a significant enhancement in AMP-stimulated NE release in this brain region. These results are consistent with the idea that alterations in NE neurotransmission may contribute to both post-AMP withdrawal 'behavioral depression' and the persistent effects of AMP on behavior (i.e. behavioral sensitization).

822.7

ALPHA-2 ADRENERGIC FUNCTION AND VISUAL SELECTIVE ATTENTION IN MONKEYS.
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Catecholinergic innervation of the cerebral cortex may have an important function in controlling attention. The well documented ability of alpha-2 agonists to improve working memory may, at least in part, be due to the memory being protected from irrelevant or distractive stimulation (Arnsten & Contant, *Psychopharmacology*, 180:159-169, 1992). This indicates that alpha-2 agonists may also have a beneficial effect on attentive functions. In the present study the monkeys (*Macaca arctoides*) were trained to perform spatially selective manual responses (left/right) in a task in which the stimulus colour (green/red) and its spatial location (left/central/right) were relevant and irrelevant stimulus dimensions, respectively. The location of the colour stimulus was compatible, incompatible or neutral with respect to the direction of the response. The different types of stimulus conditions were presented in random order within the block of testing. The effects of medetomidine, an alpha-2 agonist, and its antagonist atipamezole were studied by comparing their effects on saline control performance separately in each stimulus condition. The role of alpha-2 adrenergic function will be discussed in the context of visual selective attention.

822.8

STIMULATION OF MEDIAL SEPTAL NORADRENERGIC β-RECEPTORS INDUCES BEHAVIORAL AND FOREBRAIN EEG ACTIVATION.
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We previously demonstrated that acute, selective manipulations of locus coeruleus (LC) neuronal activity elicit robust alterations in forebrain EEG in halothane-anesthetized rat. Similar EEG responses are observed following small infusions of noradrenergic β-agonists and antagonists into the basal forebrain region encompassing the medial septum and vertical limb of the diagonal band of Broca (MS) in anesthetized rat. The present studies assessed whether the EEG activational effects induced by activation of MS β-receptors are observed in the absence of anesthesia, and if so, whether concomitant behavioral effects are observed. Rats were implanted with a guide cannula aimed at MS or adjacent regions. On the day prior to testing, rats were singly housed in sound attenuated chambers. On the day of testing, a 33 ga. infusion needle was inserted and attached to the cannula via plastic threaded sleeves and videotaping of the rat's behavior began 30 min later. 60 min following the onset of behavioral recording, an infusion (150 nl) of either vehicle or the β-adrenergic agonist isoproterentol (ISO; 25 ug/ul) was made without disturbing the animal. EEG was simultaneously recorded in a subset of animals. MS vehicle infusions, or ISO infusions, placed outside MS did not alter EEG measures or the amount of time spent awake compared to the preceding hour. In contrast, MS ISO infusions resulted in hippocampal/cortical EEG activation and within 3-10 min, increased the time spent awake in the hour following the infusion (5 vs. 34 min). EEG responses preceded the behavioral responses and were present at the latter portions of the behavioral response which were often characterized by brief and frequent bouts of lying down interrupted by shifts of body position and brief periods of locomotor activity. These results indicate that the LC-noradrenergic system exerts an excitatory influence on behavioral and EEG measures of arousal in the unanesthetized rat, via actions within MS.

822.9

IMPROVED VIGILANCE PERFORMANCE IN MONKEY ASSOCIATED WITH ALTERED LOCUS COERULEUS (LC) ACTIVITY AFTER CLONIDINE.
J. Rajkowski*, S. Ivanova, P. Kubiak & G. Aston-Jones, Dept. Psychiatry, Hahnemann Univ., Philadelphia, PA 19102.

The effect of the alpha2 agonist, clonidine (5, 20 or 40 ug/kg), on activity of LC neurons was studied in 4 Cynomolgus monkeys while they performed a vigilance task requiring sustained focused attention (see Kubiak et. al, this volume). Behavioral effects of clonidine were dose-dependent: 5 ug/kg (im) had no effect on task performance, 20 ug/kg (im, sc) resulted in drowsiness or mild sedation with continued but improved performance, and 40 ug/kg (im) produced strong sedation.

In 3 monkeys who performed well before clonidine (< 5% errors of commission or false alarms; FAs), clonidine administration reduced errors (increased ß) but also increased bar release latency. LC discharge was unaffected by 5 µg/kg clonidine, but was consistently reduced by the higher doses. There was an inverse relationship between tonic LC activity and task performance after clonidine. However, if LC neurons became totally quiescent animals became drowsy and performance ceased.

Clonidine produced an unexpectedly strong effect in the fourth monkey. This animal exhibited hyperactive behavior throughout training (FAs ~ 30%). Clonidine (20 µg/kg, sc) produced prolonged epochs of near-perfect performance (5% FAs; increased ß and d'), alternating with epochs of drowsiness. By 2 h after clonidine, performance had deteriorated to preinjection levels and motor hyperactivity returned. Overall, task performance correlated strongly with the level of LC tonic activity: Whereas poor performance before clonidine invariably corresponded to elevated tonic LC activity, improved performance after clonidine was associated with low-frequency LC discharge. Phasic LC responses to CS+ cues, typically small in this monkey, increased markedly after clonidine.

These results support other findings of improved sustained attention after clonidine, especially notable in hyperactive animals, and suggest the possibility that alterations in LC activity may be a causal factor. Clonidine will be microinfused into the LC to selectively inhibit LC neurons as a test of this hypothesis. Supported by AFOSR grant F49620-93-1-0099.

822.10

CONTRASTING RESPONSES OF MONKEY LOCUS COERULEUS (LC) NEURONS TO CONDITIONED VS. UNCONDITIONED STIMULI: RELATIONSHIP TO AROUSAL, PERFORMANCE AND VIGILANCE.
P. Kubiak*, S. Ivanova, J. Rajkowski and G. Aston-Jones. Div. Behav. Neurobiol., Dept. Psychiatry, Hahnemann Univ., Philadelphia, PA 19102

We previously reported that performance degrades with prolonged behavior on a vigilance task, and varies over shorter epochs within the task, in association with altered LC responses to CS+ cues. We now report that LC responses to conditioned vs. unconditioned stimuli exhibit converse changes during naturally occurring drowsiness, or during inattentiveness associated with high arousal. LC neurons were recorded from 3 Cynomolgus monkeys performing a vigilance task. Animals were required to foveate a central fix spot to initiate each trial of stimulus presentation; such foveation ensured attentiveness to the task. Animals were trained to release a pedal after infrequent CS+ stimuli and withhold responding to frequent CS- stimuli. LC neurons were phasically activated at a short latency (~120 msec) by CS+ stimuli but not by other task events. During occasionally occurring periods of drowsiness task performance deteriorated, as evidenced by decreased frequency of foveation of the fix spot, increased errors of omission, and longer latencies of lever release. During drowsiness the tonic discharge rate of LC neurons decreased and LC responses to CS+ stimuli were significantly smaller. In contrast, LC responses to unconditioned auditory stimuli (white noise bursts; 70 msec, 75 db) were of greater magnitude during drowsiness and poor task performance. Similarly, LC responses decreased for CS+ cues, but increased for unconditioned stimuli, during epochs of inattentiveness and poor performance associated with high arousal and apparent agitation. These results reveal that (1) a close relationship exists between attentiveness, LC tonic and phasic activities, and performance of a vigilance task, (2) LC responses to CS+ cues decrease during inattentiveness associated either with drowsiness or with high arousal and agitation, and (3) responsiveness of LC neurons to unconditioned stimuli is magnified during these same periods of inattentiveness. Our findings indicate that LC responsiveness depends on the state of attention and support the role of LC in attention and vigilance. Supported by AFOSR grant F49620-93-1-0099.

822.11

A COMPUTATIONAL MODEL OF LOCUS COERULEUS INFLUENCE ON PERFORMANCE IN A VISUAL DISCRIMINATION TASK

M. Usher, J.D. Cohen, D. Servan-Schreiber, R. Zemel*, Dept. Psychology, Carnegie Mellon Univ., Pittsburgh, PA 15213, P. Kobiak, J. Rajkowski and G. Aston-Jones, Hahnemann Univ.

A computational model is proposed that accounts for the relationship between patterns of tonic and phasic Locus Coeruleus (LC) activity and behavioral performance during a vigilance task: improved performance during periods of lower tonic activity together with phasic bursts after target stimuli. The model shows how the relationship between patterns of LC firing and behavioral performance can be explained by changes in electrotonic coupling within LC. Specifically, it demonstrates that an increase in electrotonic coupling results in a more synchronous (i.e., phasic) pattern of firing that is triggered by target but not by distractor stimuli, and that is also associated with a lower spontaneous (i.e., tonic) firing rate. This results in tighter coupling of neocortical NE release to target stimuli, which in turn improves task performance (a reduction in false alarms without an increase in response latency to targets). The model provides an account of the influence of LC cells and NE on performance in a cognitive task, suggesting a specific role for electrotonic coupling within LC and neuromodulatory influence of NE in selective attention.

822.12

IS A CONNEXIN INVOLVED IN NEURAL CONTROL OF LUMINESCENCE IN THE CNIDARIAN *RENILLA KOELLIKERI*? G. Germain and M. Anctil*. Dept. Sci. Biol., Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.

Of the three classes of the phylum Cnidaria, Hydrozoa but neither Scyphozoa nor Anthozoa possess ultrastructurally identifiable gap junctions. We present evidence that the bioluminescence system of an anthozoan, the sea pansy, which is believed to be coordinated by a nerve-net but which is also adrenergically controlled via cells neighboring the light-emitting cells (photocytes), requires gap junction-like coupling to effect light emission. Gap junction blockers such as octanol and heptanol reversibly eliminated in situ luminescent responses but not those of dissociated photocytes. An anti-connexin43 monoclonal antibody (anti-Cx43) loaded in permeabilized cells abolished the luminescent responses. Western blot analysis revealed the presence in sea pansy tissues of a Cx43-like protein. We also visualized Cx43-like punctate immunoreactivity in some epithelial layers and in the nerve-net, with greater abundance in the luminescent endoderm. Ultrastructural observations revealed only small "close appositions" but not recognizable gap junctions. These results suggest that a Cx43-like gap junction protein exists in the sea pansy and is involved in transmission of bioluminescent signaling, but does not aggregate in typical large plaques. (Supported by NSERC)

822.13

THE ALPHA1-ADRENOCEPTOR ANTAGONIST PRAZOSIN ANTAGONIZES MK-801 EVOKED STIMULATION OF MESOLIMBIC DOPAMINE ACTIVITY: ELECTROPHYSIOLOGICAL, BIOCHEMICAL AND BEHAVIORAL CORRELATES J.M. Mathé, G.G. Nomikos, B.E. Hildebrand, K. Chergui, P.Hertel, A.A. Mathé' and T.H. Svensson Dept. Physiology & Pharmacology, Div. Pharmacology, Karolinska Institutet, 171 77 Stockholm, SWEDEN.

The effects of the potent alpha-1-adrenoceptor antagonist prazosin were investigated on electrophysiological, biochemical and behavioral action of the psychotomimetic, NMDA-receptor antagonist MK-801 related to the mesolimbic dopamine (DA) system in the rat.

Extracellular single-cell recordings were obtained from A10 DA neurons in chloral hydrate anaesthetized rats. Action potentials were fed into a computer for analysis of firing rate, burst firing and regularity of firing, as assessed by the variation coefficient. In addition, levels of DA and its metabolites were measured in the n. accumbens (NAC) with microdialysis in freely moving rats. Locomotor activity was assessed in rats in an open field. Rats were pretreated with either vehicle or prazosin (0.3-1.0 mg/kg), 20 min prior to MK-801 (0.05-0.3 mg/kg) administration.

Administration of prazosin 1) effectively blocked the increased burst firing, but did not affect the average firing rate of A10 DA neurons 2) antagonized the increased DA, DOPAC and HVA levels in the NAC, and 3) blocked hyperlocomotion, as induced by MK-801.

Thus, behavioral stimulation by MK-801 is indeed associated with increased presynaptic activity in the mesolimbic DA system. Moreover, a potent and selective alpha-1-adrenoceptor antagonist can specifically block this, subcortically evoked DA activity without any significant effect on basal DA release in the same region. Supported by the Medical Research Council of Sweden and the Karolinska Institutet.

822.14

LONG-TERM EFFECTS OF PSYCHOMOTOR STIMULANTS ON APPETITIVE AND CONSUMMATORY SEXUAL BEHAVIORS IN THE MALE RAT. M.F. Wilkins*, L. Schattmann, and J.G. Pfaus. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, QC, Canada.

Amphetamine and cocaine are generally believed to enhance human sexual desire, arousal, and performance, whereas long-term use of these drugs is associated with a deterioration of sexual function. Surprisingly, these effects have not been studied extensively in animals. We examined the acute and long-term effects of cocaine hydrochloride (5, 10, or 20 mg/kg) or *d*-amphetamine sulfate (1.25, 2.5, or 5 mg/kg) on sexual behavior in sexually experienced male rats. Amphetamine or cocaine were injected prior to tests conducted every 4 days in bilevel chambers. Acute amphetamine increased anticipatory level changes, but decreased the proportion of rats that mounted, intromitted and ejaculated. Long-term amphetamine decreased anticipatory level changes secondarily to the induction of stereotypy, and also further decreased the proportion that mounted, intromitted or ejaculated. These effects reversed during withdrawal, but declined following challenge dosages of amphetamine. In contrast, acute cocaine did not affect anticipatory level changes or the proportion of rats that mounted, intromitted or ejaculated. However, cocaine increased mount and intromission latencies, decreased ejaculation latencies, and decreased the number of intromissions before ejaculation. Although tolerance accrued to these effects, challenge dosages after withdrawal further reduced the number of intromissions and the ejaculation latencies. Thus, acute and long-term administration of amphetamine or cocaine differentially affect sexual behavior in the male rat.

NEUROPEPTIDES AND BEHAVIOR III

823.1

THE EFFECT OF NPY ADMINISTERED TO EITHER THE VMN OR THE PVN ON LORDOSIS AND FEEDING. C. Bauer and J.E. Thornton*, Neuroscience/Biopsychology Program and Department of Biology, Oberlin College, Oberlin, OH 44074

Recently we have shown that neuropeptide Y (NPY) increases lordosis in female guinea pigs. The effect of NPY on feeding in guinea pigs has not been examined. The present experiment determined whether NPY might act on either the ventromedial nucleus (VMN) and/or the paraventricular nucleus (PVN) of the hypothalamus to affect lordosis and feeding in female guinea pigs.

Ovx female guinea pigs had a cannula implanted into either the VMN or the PVN. For lordosis tests, estrogen-and progesterone-primed females were infused with 100 pmol NPY or saline vehicle in a repeated measures, counterbalanced design, and tested for lordosis behavior. NPY significantly increased lordosis when it was administered to the VMN but not the PVN. For feeding tests, ovx females were infused with 100 pmol NPY or vehicle and feeding was examined. NPY administered to either the PVN or the VMN significantly increased both the number of grams eaten and the frequency of feeding. It is suggested that NPY acts at the VMN to affect lordosis and at both the VMN and the PVN to affect feeding in the female guinea pig.

823.2

DRINKING INDUCED BY MICROINJECTIONS OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP38) INTO THE LATERAL HYPOTHALAMUS (LH) OF NONDEPRIVED RATS. M.Puig de Parada*, M.A.Parada, and L.Hernández. Department of Physiology, School of Medicine, Universidad de los Andes, Mérida 5101-A, Venezuela.

PACAP38 increases cAMP levels in rat pituitary cells and sulpiride increases cAMP in neurons bearing dopamine D2 receptors. Sulpiride induces also several behaviors when bilaterally administered in the perifornical LH (pfLH). The present study tested if bilateral pfLH PACAP38 microinjections could reproduce the behavioral effects of sulpiride. PACAP38 (1 nmol/0.5 µl) administered in the vicinity of the pfLH selectively induced drinking (19.7±4.1 ml of water) during the hour following the injection. In the same rats (n=12) sulpiride (45 nmol/0.5 µl) had milder effects (7.8±1.4 ml/hr). The difference between both effects was statistically significant ($t = 3.61$; $df=11$; $p<0.005$). Neither sulpiride nor PACAP38 promoted drinking when injected (n=10) 1.3 mm behind the effective zone. This negative result is an evidence of the neuroanatomical specificity of the dipsogenic effect of both drugs. The dipsogenic effects of sulpiride and PACAP38 were well correlated [$R = 0.821$; $F(1/20) = 41.5$; $p<0.0001$] suggesting that both substances trigger drinking by activation of the same hypothalamic mechanisms. These results suggest that PACAP38 in the pfLH could be an integrative neuropeptide regulating drinking behavior.

823.3

CHOLECYSTOKININ ATTENUATES GROOMING VIA AN ATYPICAL RECEPTOR TYPE. J.M. Van Kampen* and A.J. Stoessl. Clin. Neurol. Sci., University Western Ontario, Ont., Canada.

The neuropeptide cholecystokinin (CCK) is known to interact with dopamine in various ways. We have demonstrated that CCK-8S effectively attenuates dopamine D1-mediated vacuous chewing movements (VCM's) and stereotypic grooming. Our work has shown a clear role for the CCK-A receptor in the attenuation of **VCM's** but the receptor type mediating this effect on **grooming** remains unclear. While a selective CCK-B agonist was effective in attenuating grooming, the CCK-A rather than the CCK-B antagonist blocked the attenuating effects of a general agonist. Similar anomalous results have been found by others in studies of anxiety and analgesia. To further explore this issue we examined the effects of selective CCK antagonists on the attenuation of grooming by a CCK-B agonist. Administration of SKF 38393 (5mg/kg,s.c.) to male Sprague Dawley rats resulted in a significant increase in grooming which was reduced to control levels by the peripheral administration of the selective CCK-B agonist CCK-4 (20µg/kg,i.p.). Pretreatment with **both** the CCK-A antagonist, MK-329, and the CCK-B antagonist, L-365,260, significantly blocked this attenuation over a range of doses (20µg/kg,100µg/kg, 500µg/kg;i.p.). SKF 38393-induced VCM's were unaffected by CCK-4 or either of the antagonists. Further study has ruled out possibile influence from anxiogenic properties of CCK-4 or direct effects of the antagonists themselves. We also examined the effects of vagotomy on the actions of CCK-8S. Vagotomy abolished suppression of both VCM's and grooming. Taken together, these findings suggest a predominant peripheral 'A' receptor mediated effect of CCK but CCK-4 may be acting at a novel receptor subtype.

823.4

FETAL MOTOR BEHAVIOR AND RESPONSIVENESS TO PERIORAL CUTANEOUS STIMULATION: INVOLVEMENT OF V1 RECEPTORS. E.I. Varlinskaya & E.S. Petrov, Inst. for Exptl. Med., St. Petersburg, Russia and W.P. Smotherman*, Ctr. Dev. Psychobiol., Binghamton University - SUNY, Binghamton, NY.

Arginine-vasopressin (AVP) injected into the cisterna magna of the E20 rat fetus increases motor activity, including the relatively uncommon patterns of mouthing, licking and facial wiping. The AVP effect is mediated by V1 receptors in the brain stem and spinal cord. The effect of AVP on fetal motor behavior can be potentiated by intrahemispheric (IH) injection of the V1 antagonist [ß-mercapto-ß,ß-cyclopentamethylene-propionyl^1,O-Me-Tyr^2,Arg^8]-vasopressin. These findings suggest that there are at least two populations of V1 receptors: one in the spinal cord/brain stem that has an excitatory effect and a second in the brain hemispheres that has an inhibitory effect on fetal motor behavior. Fetal responses to perioral cutaneous stimulation including the presentation of an artificial nipple also are influenced by manipulation of V1 receptors in the spinal cord/brain stem and brain hemispheres. IC injection of the V1 antagonist reduces fetal responsiveness while blockade of V1 receptors by IH injection has the opposite effect and potentiates responsiveness to perioral cutaneous stimulation including oral capture and grasping of the artificial nipple. These findings indicate that AVP plays a role in regulating fetal motor behavior including responsiveness to perioral cutaneous stimulation and suggests that AVP may affect behavior immediately after birth in the context of suckling.

WPS is supported by an MERIT Award from NIH (HD 16102-11).

823.5

NO EFFECT OF CENTRALLY ADMINISTERED CCK-8S ON RETENTION OF INHIBITORY AVOIDANCE IN FEMALE RATS AS A FUNCTION OF ESTRUS STATE. L. J. Wichlinski*, C. Dietrich, A. Mayer, C. Wesely, L. Flaten, B. Hogan, C. Kulawik, A. Sparks, M. Cervera, and M. Dedricks. Dept. of Psychology, Carleton College, Northfield, MN 55057.

In previous experiments from our laboratory, centrally administered cholecystokinin (CCK)-8S produced a significant enhancement of retention performance in the one-trial inhibitory avoidance task in male but not female rats. In this experiment we sought to determine the role of the estrus cycle in the failure of CCK-8S to produce memory enhancement in female rats. Adult female Sprague-Dawley rats were implanted stereotaxically with guide cannulae one week before training. Immediately following training on the one-trial inhibitory avoidance task (0.6 mA, 1.0 sec) rats were infused i.c.v. with either CCK-8S (800 pmoles) or vehicle. Testing took place 24 hr later. Rats were assigned to one of four estrus states (metestrus, pro-estrus,estrus, or diestrus) based on light microscope assessments of vaginal epithelial cells collected prior to training. Behavioral results were matched to estrus groups in a blind fashion after testing. Again, CCK-8S failed to enhance retention performance relative to vehicle treatment, regardless of estrus state. Also, vehicle results alone did not vary as a function of estrus state. Possible reasons for the absence of CCK-8S effects include: insensitivity of the task to CCK-8S modulation in female rats, a low threshold for suppression of CCK-8S by circulating ovarian steroid hormones, differential dose-response curves for female and male rats, and interactions between CCK-8S and testosterone. Ovariectomy experiments are in progress to explore this issue further.

823.6

MODULATION OF CORTICOTROPIN-RELEASING FACTOR (CRF) RELEASE BY NMDA AND 5HT$_{1a}$ RECEPTORS IN PRIMARY CULTURES OF FETAL RAT AMYGDALA. D.L. Birkle*, V. Suski, M.S. Cratty, R.W. Gabr, H.A. Mason, M.G. Villanueva, A.K. Salm. Department of Pharmacology and Toxicology, Department of Anatomy, WVU School of Medicine, Morgantown, WV 26506.

Previous immunocytochemical studies from our laboratory have demonstrated the presence of corticotropin-releasing factor (CRF)-positive neurons in primary cultures of fetal rat amygdala neurons. We have also previously shown Ca^{2+}-dependent, depolarization-induced release of CRF in these preparations, and CRF release in response to the excitatory amino acid neurotransmitter, glutamate. We now have evidence that glutamate-induced CRF release is mediated primarily by activation of NMDA receptors. NMDA (EC_{50}, 5 µM) stimulated CRF release. The effects of both glutamate and NMDA were blocked by AP-5 (IC_{50}, 30 µM), or by restoring $[Mg^{2+}]_{out}$ to 1 mM. Kainic acid (EC_{50}, 0.5 µM) also stimulated CRF release, and was partially antagonized by AP-5, suggesting that its effects are mediated in part by the release of glutamate in the cell culture. Activation of 5-HT$_{1a}$ receptors with the agonist, 8-OH-DPAT, reduced both basal and depolarization-induced (60 mM K^+) release of CRF (EC_{50}, 1 nM). These studies demonstrate stimulatory (via NMDA receptors) and inhibitory (via 5HT$_{1a}$ receptors) modulation of CRF release from fetal amygdala neurons in primary culture. Supported by NSF (IBN-9222263).

823.7

FORCED-SWIM TEST REDUCES CYCLO(HIS-PRO) LEVELS IN RAT BRAIN. A.E. Pekary*, R.L. Lloyd, M. Chilingar, A. Sattin. Psychiatry, Medicine (Endocrinology) & Research Services, West Los Angeles VA & UCLA School of Medicine, Los Angeles, CA 90073.

Electroconvulsive seizures (ECS) elevate the levels of both thryotropin releasing hormone (TRH) and TRH-Gly (pGlu-His-Pro-Gly), a TRH precursor, in the limbic system of rats. We have reported significant correlations between results from the forced swim test (a test used to predict efficacy of antidepressant drugs and ECS) and post-ECS TRH and TRH-Gly levels in four anterior limbic regions dissected from male Wistar rats (Ann NY Acad Sci, 739:135, 1994). Because TRH and TRH-Gly are readily converted to cyclo(His-Pro) (cHP), we have studied the effects of ECS and forced swimming on the brain levels of this metabolically stable cyclic dipeptide. cHP has a number of CNS effects including the induction of hypothermia and suppression of motor activity which may influenced, and in turn be influenced by ECS and forced swim. The rat groups were: untreated controls, swim only, ECS only, and swim+ECS. Below are the cHP levels (ng/g wet wt.) for pyriform ctx. (PYR), striatum (STR) and anterior ctx. (AC)(mean ± SD):

	Control + ECS only (18)	Swim only + (Swim+ECS) (16)	p
PYR	60.9 ± 29.5	39.4 ± 11.1	<0.01
STR	46.6 ± 19.6	29.7 ± 9.6	<0.004
AC	54.9 ± 57.3	29.1 ± 13.3	n.s.

Forced swim testing significantly decreased cHP in 2 of the 3 regions tested. ECS did not affect mean cHP levels in these regions (not shown). Mechanisms of this effect are unknown. It is known that cHP can be derived from sources other than TRH. We thank Dr. Ivor Jackson for the cHP antiserum. Supported by VA Research Service.

823.8

THE EFFECTS OF NGF, b-FGF AND CEREBROLYSIN ON SPATIAL NAVIGATION AFTER BILATERAL LESIONS OF THE SENSORIMOTOR CORTEX: A. Gschanes, V. Valouskova, M. Windisch and H. Xiong*. Center of Animal Biology, Medical School, Roseggerweg 48, A-8036 Graz, Austria.

Damage of the sensorimotor cortex causes spatial navigation deficits in rats. Lesions of this brain region were made with suction and the subsequent behavioral effects measured in a Morris water maze apparatus. bFGF, NGF or Cerebrolysin (CER) a nootropic peptidergic drug, were infused by osmotic minipumps unilaterally into cavities made by suction or intraperitoneally (ip). For comparison, other unlesioned rats were injected ip with saline or CER. Animals were tested in the water tank 13 days after surgery for 3 consecutive days. The length of the trajectory to a submerged platform, as well as the speed of swimming, were measured. The trajectory of lesioned animals was significantly longer than of controls but none of the treatments changed this parameter. However, all groups treated ip and the NGF group infused centrally swam faster towards the platform in comparison to the corresponding lesioned or intact controls. The same animals were evaluated 8 months after treatments. Only FGF-treated animals showed a significant improvement in comparison to the lesioned controls. We conclude that, in the short-term, CER improves spatial memory in lesioned and intact animals whereas FGF leads to more long-lasting effects.

823.9

CONVERGENT MODULATION OF A NEUROMUSCULAR JUNCTION BY NINE NEUROHORMONES. J.C.Jorge-Rivera* and E.Marder, Volen Center, Brandeis University, Waltham, MA 02254

Despite numerous examples of invertebrate neuromuscular junction (NMJ) modulation by amines and peptides, little is known about the physiological organization of NMJ modulation by multiple substances.

We have found that a stomatogastric NMJ in the crab Cancer borealis is modulated by 9 neurohormones: Red Pigment Concentrating Hormone (RPCH), Proctolin (PROC), TNRN- and SDRN-FLRFamide, Serotonin (5-HT), Octopamine (OCT), Dopamine (DA), D-Allatostatin-3 (D-AST-3), and Histamine (HIS). We studied the effect of each modulator on agonist-mediated contractions, nerve-evoked contractions, high K^+ contractions, nerve-evoked excitatory junctional potentials (EJPs), and excitatory junctional currents (EJCs). Changes in muscle fiber input resistance were also investigated in current clamp and voltage clamp conditions. While 7 modulators enhanced the efficacy of this NMJ, 2 of them were inhibitory. For example, PROC enhances nerve-evoked muscle contraction by 500%±100% (n=3) whereas D-AST-3 reduces contractions by 60%±7% (n=7). It is likely that this NMJ is targeted by different complements of modulators under different physiological contexts in vivo. Pairs of modulators (RPCH and PROC, 5-HT and AST, FLRFamides and HIS) produce different responses when coapplied than when either is applied alone.

Supported by NS17813 (EM) and Ford Foundation Dissertation Award (JCJR).

823.10

INCREASES IN CYCLIC-GMP OCCUR AT ECDYSIS IN AN EVOLUTIONARILY CONSERVED INSECT NEURONAL NETWORK. J. Ewer and J.W. Truman*, Zoology Dept., U. of Washington, Seattle, WA 98195.

In the moth *Manduca sexta*, the neuropeptide eclosion hormone (EH) is released at the end of each molt and triggers ecdysis, the shedding of the old cuticle. Just in anticipation of ecdysis a network of 25 pairs of peptidergic neurons shows a dramatic increase in cGMP-immunoreactivity (cGMP-IR; Ewer et al.(1994) J. Neurosci., **12**: 7704-7712). This network includes 5 pairs of neurons in the subesophageal ganglion, and two pairs in each of the thoracic and first 7 abdominal ganglia. In moths the abdominal neurons contain Crustacean CardioActive Peptide (CCAP).

A homologous neuronal network becomes cGMP immunoreactive during ecdysis in a variety of insects. In the most ancestral insects, such as silverfish (Zygentoma) and grasshoppers (Orthoptera), some of the abdominal CCAP neurons are present but do not show the cGMP response. Other insects like crickets (Orthoptera), meal worms (Coleoptera), and mosquitoes (Diptera) have the full complement of 50 cells participating at ecdysis. Higher Diptera (*Drosophila, Sarcophaga, Musca*) are exceptional in that the ecdysial cGMP response is absent although most, if not all, of the CCAP neurons are present. We will discuss how variation in the pattern of cGMP expression in this network may relate to differences in ecdysis behaviors.

823.11

AMPLITUDE REDUCTION OF EVOKED HIPPOCAMPAL FIELD POTENTIALS DURING SEXUAL BEHAVIOR IN THE FEMALE RAT. Grossman, K., Burstein, U., Zeppelin, D., Ward, T., Sween, K., Brittin, L., Carder, S., Kelley, S., Wolfson, W., Smock, T*. Behav. Neuroscience Prog., Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

These studies were designed to investigate the influence of sexual behavior on the amplitude of evoked hippocampal field potentials. Ovariectomized Sprague- Dawley rats underwent chronic electrode implant surgery. The stimulating electrode was placed in the stratum radiatum (near CA3) and the recording electrode was placed in CA1. Biphasic 0.5ms constant current square wave pulses were delivered every five seconds. Evoked potentials (EP) were reduced 30% below maximum and recorded before (B), during (S) and after four testing conditions (TC): sexual-social (receptive fem. +male, n=10); non-sexual social (non-receptive fem.+ male, n=8 & non-receptive fem.+ fem. n=11); and non-sexual non-social (non-receptive fem.+ food, n=8). Amplitude reduction (AR) during the TC was measured as the change in the average amplitude between B and S. AR for specific behaviors (i.e. locomotion, grooming) was measured as the change in the average amplitude between B and the behavior. The results suggest that there may be a greater AR in the sexual-social TC during the overall S period compared to the AR in the other three TC. There was a significantly greater AR during locomotion in the sexual-social TC compared to the receptive fem.+male TC. However, there were no differences in AR during locomotion compared to other specific behaviors within TC. AR during locomotion is attributed to the difference between the highest and lowest locomotion AR. The results suggest that the females in the sexual social TC may be non-specifically more aroused than in the other TC and that the AR may reflect this difference.

823.12

PROFILE OF CGRP-, AMYLIN- AND SALMON CALCITONIN-INDUCED DEPRESSION OF LOCOMOTION FOLLOWING INTRACEREBRO-VENTRICULAR INJECTIONS D. van Rossum*[1,2] and R. Quirion[2]. [1]Max-Planck Institute for Brain Research, 60528 Frankfurt/M, Germany, [2]Douglas Hospital Research Centre, McGill University, Montréal, Canada, H4H 1R3.

Calcitonin gene-related peptide (CGRP)-, amylin- and calcitonin-like immunoreactivity, as well as respective binding sites, are widely distributed in the CNS. Few brain areas, including the nucleus acumbens, are enriched with binding sites for all three peptides (van Rossum *et al., J. Pharmacol. Exp. Ther.* **270**, 1994). The central administration of either CGRP, amylin or calcitonin induces specific neurobehavioral profiles. In the present study, we investigated the effects of each of these peptides on amphetamine-induced (2 mg/kg) as well as on spontaneous locomotor activities. Locomotion was recorded by photocell interruptions in activity chambers for 3 hrs following animal treatment. Intracerebroventricular injections of each peptide (0.04-20 µg/10µl) decreased, in a dose dependent manner, the amphetamine-induced locomotion. All three peptides showed similar maximal effect (~ 40 %) in decreasing amphetamine-induced locomotion. However, salmon calcitonin revealed to be most potent (0.16 µg) followed by rat amylin (1.25 µg) and human CGRPα (hCGRPα, 2.5 µg). A similar potency profile was observed on spontaneous locomotion. Interestingly, the injection of bicuculline (0.5 mg/kg), a $GABA_A$ receptor antagonist, selectively blocked the decrease in spontaneous locomotion induced by hCGRPα while having no significant effects on rat amylin and salmon calcitonin-induced decrease in spontaneous locomotor activity. The present findings thus support the existence of functionally distinct receptor subtypes for CGRP *vs.* those of amylin and salmon calcitonin to modulate locomotor activity in the rat brain. Supported by the MRC of Canada.

823.13

CONDITIONED BLOOD GLUCOSE CHANGES IN HUMANS: CENTRAL INSULIN EFFECT OR PERIPHERAL EFFECTOR? G. Fehm-Wolfsdorf*, J. Pohl and W. Kerner. Institute of Psychology, University of Kiel, D-24098 Kiel, Germany.

Classical conditioning of changes in blood glucose level has been repeatedly demonstrated in animal experiments, and more recently in human studies (Fehm-Wolfsdorf et al., 1993, Physiol Behav, 54,155). In their model of drug-induced conditioning Eikelboom & Stewart (1982, Psychol Rev, 89,507) discuss two possibilities for the unconditioned stimulus and response: in glycemic conditioning they consider a direct action of insulin on a peripheral effector mechanism or a CNS mediated activation of effectors. The present study aimed to compare these possibilities directly within a yoked-control design: the "peripheral group" (PG) was infused with insulin (infusion rate 1.5 mIE/kg/min) until they clearly noticed symptoms of hypoglycemia (mean duration 24 min), then the CS (a strange mixture of odours) was given for 10 min while blood glucose was held constant by a clamp technique (group mean 2.3mM/L). Subjects of the "central group" (CG) were matched with the subjects of the PG relative to the decrease of blood glucose following insulin injection in a previous matching session. During the acquisition sessions the yoked partner in the CG was infused with the identical amount of insulin, but in addition received glucose to maintain stable blood glucose levels (group mean 5.1mM/L). The CS was given to the CG at the hypothetical time of a central insulin effect, i.e. 5 min after start of infusion for 10 min. 30 male healthy drug-free non-smoking volunteers participated in 6 sessions each: a matching session, 4 conditioning sessions and a test session with saline infusions for all subjects. Within the test session a CR was defined as a change from baseline by >0.45mM/L blood glucose. In the PG 40% and in the CG 47% of the ss showed a CR, half of them hypo- or hyperglycemia. From this result we would conclude that conditioning occurs as an effect of insulin and results in a disturbance of homeostatic regulation of blood glucose. Research was supported by a grant from the DFG.

823.14

INHIBITION OF NOVELTY-INDUCED LOCOMOTOR ACTIVITY BY INTRA-VTA INJECTION OF NEUROPEPTIDE FF, AN ANTI-OPIOID PEPTIDE. Marco N., Cador M., Stinus L.*, Le Moal M. and Simonnet G. INSERM U.259 - Rue Camille Saint-Saëns 33077 Bordeaux, France

The neuropeptide FF (NPFF) is a mammalian neuropeptide with some antiopioid properties such as anti-analgesic effects. Brain NPFF receptors are different from opioid receptors and are present at the Ventral Tegmental Area (VTA) level on non-dopaminergic cells. We have previously shown that intra-VTA injection of NPFF was able to inhibit intra-VTA morphine-induced locomotor activity. Thus, the presence of both NPFF and opioid systems at the VTA level suggest that these two opposing systems may participate to a physiological regulation of the activity of the mesocorticolimbic dopaminergic system. To test this hypothesis, we have studied the locomotor activity elicited by a novel environment since it has been reported that the mesocorticolimbic dopaminergic system is required for the expression of novelty-induced locomotor activity. In this study, we have shown that intra-VTA injection of naloxone methobromide induced a biphasic inhibition of the locomotor activity triggered by novelty, indicating that opioid peptides are released when rats are placed into a novel environment. During an early phase (0-30 min) naloxone only partially inhibited novelty-induced locomotor activity (-30%), indicating the existence of both opioid and non-opioid components. During a second period (30-90 min) naloxone totally blocked novelty-induced locomotor activity showing an essential role for opioids at the VTA level. When injected in the VTA, NPFF inhibited in a dose dependent manner (1 to 10 µg/0.5 µl) the novelty-induced locomotor activity. The highest dose (10 µg) totally blocked the hyperactivity induced by novelty. These results indicate that this neuropeptide has both opioid and non-opioid blocking effects at the VTA level suggesting that NPFF may be a modulator of the mesocorticolimbic dopaminergic system activity.

824.1

ALCOHOL-INDUCED BEHAVIORAL DEFICITS AND REGIONAL BRAIN WEIGHT REDUCTIONS DEPEND ON TIMING OF ALCOHOL EXPOSURE DURING THE NEONATAL BRAIN GROWTH SPURT IN RATS. J.D. Thomas[1]*, C.M. Esparza[2], D.E. Moritz[2], G. Lopez[2], E.A. Wasserman[1] & J.R. West[2]. [1]University of Iowa, [2]Texas A & M University

Prenatal exposure to alcohol can produce permanent CNS dysfunction expressed as a wide spectrum of behavioral deficits; however, the nature and severity of this dysfunction varies greatly. Developmental timing of alcohol exposure is believed to be one important determinant of alcohol's teratogenic effects. Using a rat model system, the present study investigated the behavioral and CNS consequences of brief episodes of alcohol exposure during varying periods within the neonatal brain growth spurt, a time of rapid CNS development equivalent to the human third trimester. Sprague-Dawley rats were exposed to alcohol (6.6 g/kg) in a binge-like manner on two consecutive days via an artificial rearing procedure. One group was exposed to alcohol on postnatal days (PD) 4 and 5, another group on PD 6 & 7, and a third group on PD 8 & 9. A fourth group served as a calorically matched, artificially reared control, and a fifth group comprised normally reared suckle controls. Subjects were tested from PD 25-43 on a series of behavioral tasks including a motor task and a reversal training learning task. All alcohol-exposed groups were significantly impaired on the motor task; however, early exposure (PD 4/5) produced more severe deficits than later exposure. An opposite pattern for deficits was observed on the learning task. On this behavioral measure, severe deficits were observed in the groups following late exposure to alcohol (PD 6/7 & PD 8/9), but not earlier. This dissociation in the temporal pattern of behavioral vulnerability corresponded with the temporal vulnerability of regional brain weight reductions. Reductions in cerebellar weight, an area involved in motor performance, were most severe following early alcohol exposure (PD 4/5), whereas reductions in forebrain weight, which includes areas involved in cognitive tasks, were most severe following later exposure (PD 6/7 and PD 8/9). Thus, the pattern of behavioral deficits is consistent with the temporal vulnerability of various brain regions. Supported in part by NIAAA grant AA05523.

824.2

EFFECTS OF PRENATAL ALCOHOL EXPOSURE AND AGING ON AUDITORY FUNCTION IN THE RAT. M.W. Church*, E.L. Abel, J.A. Kaltenbach and G.W. Overbeck. Fetal Alcohol Research Center, Depts. Ob/Gyn and Audiology, Wayne State Univ. Sch. Med., Detroit, MI 48201.

The present study investigated select aspects of peripheral and central auditory dysfunction, as well as the pathological effects of aging, in an animal model of the fetal alcohol syndrome (FAS). Pregnant rats consumed liquid alcohol diets containing 0%, 17.5%, or 35% ethanol-derived calories, from gestation day 7 to parturition. A fourth group was untreated. Offspring of these mothers were tested for auditory and neurological function, using the auditory brainstem response (ABR) at 6, 12 and 18 months of age. The ABR provided evidence of both peripheral and central auditory disorders. Specifically, some animals in the alcohol-exposed groups showed a peripheral auditory disorder in the form of congenital sensorineural hearing loss. This was correlated with punctate lesions of and malformed stereocilia on the auditory sensory receptor cells in the inner ear. Alcohol-exposed animals also showed a central auditory processing disorder characterized by prolonged transmission of neural potentials along the brainstem portion of the auditory pathway. Animals in the highest dose group also showed an augmentation in the age-related deterioration of auditory acuity. Thus, increased peripheral and central auditory dysfunction and pathological deterioration of auditory function in old age may be sequelae of FAS. Such morbidities have important implications for the long-term clinical assessment and management of FAS patients.

Supported by NIH grant P50 AA07606.

824.3

BEHAVIORAL AND IMMUNOLOGICAL CONSEQUENCES OF BRIEF EMBRYONIC EXPOSURE TO NICOTINE OR ALCOHOL IN DOMESTIC FOWL CHICKS. R. A. Hughes[1]*, M. R. Baker[1], N. Nollen[1], S. H. Chou[2], K. A. Nordyke[2], and J. E. Cunnick[1,2]. Depts. Psychology[1], Microbiol. Immunol. Prevent. Med[2]., Iowa State Univ. Ames, IA 50010-3180.

Fertile eggs of domestic fowl (Gallus gallus) received injections into the egg air space of either nicotine tartrate (NIC; 0.0, 0.025, 0.25, 2.5, or 5.0 mg/ml/kg egg) or ethyl alcohol (ETOH; 10, 20, 30, or 40% 1 ml/kg egg) on incubation days 10-12. NIC (5.0 mg/kg) significantly ($p < .05$) affected hatch rate and hatchling survival but other NIC and ETOH amounts did not. Chick body weights at hatch were not significantly affected by either NIC or ETOH.

Indices of social distress vocalizations & activity) defensive (tonic immobility induction and duration), and immunologic responses (leukocyte proliferation to mitogen stimulation; ConA, LPS, PWM; blood thymus and spleen) were measured successively in surviving chicks at one (social measure), 14 (tonic immobility measures), and 17 (immune system measures) posthatch. On the social test neither NIC nor ETOH altered distress vocalizations but both drugs decreased activity in a dose dependent manner. On the tonic immobility test ETOH groups required fewer inductions than controls but duration was equivalent; NIC did not affect the immobility measures.

Preliminary analysis of the immune system data suggest significant effects of NIC and ETOH on leukocyte proliferation that depend, in part, upon dose and sex gender.

824.4

BINGE-TYPE ALCOHOL EXPOSURE DURING THE FIRST TWO TRIMESTERS EQUIVALENT IN THE RAT FAILED TO PRODUCE A DEFICIT IN CEREBELLAR PURKINJE CELL NUMBER USING STEREOLOGICAL METHODS. Susan E. Maier* & James R. West, Anatomy & Neurobiology, Texas A&M University Health Science Center, College Station, TX 77843-1114

Women who binge drink throughout pregnancy have an increased risk for delivering babies with Fetal Alcohol Syndrome or Alcohol-Related Birth Defects. In the rat, cerebellar Purkinje cells are extremely susceptible to alcohol-induced cell death as a consequence of third trimester equivalent exposure (postnatal days 4-9). Whether alcohol exposure during the first two trimesters equivalent results in similar Purkinje cell deficits is the question we addressed. Timed pregnant rats received one of three treatments daily from gestation days (GD) 1-20; alcohol gavage (6 g/kg, 22.5% v/v), Maltose-Dextrin gavage + pairfed to alcohol, or standard lab chow. Offspring from treated dams were perfused with fixative on GD33 (postnatal day 10), had their cerebella removed and processed for plastic embedding using Historesin®. Serial sagittal sections were cut through the entire cerebellum and mounted on slides for counting using Cavalieri's principle and the optical disector. Even though peak blood alcohol concentrations (BAC) reached 334 mg/dl late in gestation, there were no differences between the three groups in estimates of volume, density, or Purkinje cell number. Taken together with previous findings, these results suggest that cerebellar Purkinje cells are considerably less susceptible to alcohol-induced deficits during the first two trimesters equivalent than during the third trimester equivalent, even when alcohol exposure during the third trimester equivalent encompasses a substantially shorter duration (6 days) and results in a considerably lower peak BAC (~250 mg/dl). Supported by Grant AA10090.

824.5

ALCOHOL-INDUCED HIPPOCAMPAL ABERRANT MOSSY FIBER PROJECTIONS DEPEND ON THE PATTERN OF EARLY POSTNATAL ALCOHOL EXPOSURE IN RATS. D. M. Smith and C. R. Goodlett*. Dept. of Psychology, IUPUI, Indianapolis, IN 46202.

Continuous exposure to alcohol (EtOH) in artificially reared neonatal rats during the first ten postnatal days has been shown to produce aberrant mossy fiber projections into the distal intra- and infrapyramidal zone of mid-temporal hippocampal field CA3 (West & Hamre,*Dev. Brain Res.*, 1985). This experiment examined whether induction of aberrant mossy fibers depends on the daily pattern of neonatal alcohol exposure.

Harlan Sprague-Dawley rats were randomly assigned to one of the following treatments administered on postnatal days 1-9: one of three alcohol treatments (using artificial rearing methods); an artificially reared control; or a suckle control. Alcohol was given in three different patterns, as follows: Continuous 1 (a replication of the original West & Hamre treatment) received 2.8% v/v EtOH in each of 8, 15-min daily feedings (n=5); Continuous 2 received 2.8% v/v EtOH (n=3) or 3.2% v/v EtOH (n=5) in each of 12, 20-min daily feedings; Binge received 10.2% v/v EtOH in 2 consecutive of the 12, 20-min daily feedings (n=5). Artificially reared controls were given feedings calorically matched to the experimental groups (total n=11), and suckle controls (n=25) were reared normally by dams. Continuous 1 treatment produces more rapid increases in blood alcohol concentration (BAC) per feeding, compared to Continuous 2, because a larger volume of EtOH is delivered in a shorter feeding time. The Binge treatment produces a high peak BAC (~250 mg/dl) that falls to 0 before the end of the day. Rats were sacrificed on PD 45; frozen horizontal sections (35 µm thick) were processed with the Timm stain for heavy metals and evaluated microscopically for the presence of distal intra-and infrapyramidal mossy fibers.

In the Continuous 1 condition, 2 of the 5 cases were found to have aberrant mossy fiber projections (compared to 7 out of 8 in West & Hamre, 1985). None of the remaining groups had subjects with aberrant mossy fibers. These data suggest that the rapid and continuous presence of high BACs in the early neonatal period is critical to the development of aberrant mossy fiber projections. (Supported by AA09596)

824.6

ETHANOL-GABA$_A$ RECEPTOR INTERACTION IN DEVELOPING RAT CEREBELLAR PURKINJE CELLS: γ2L vs. γ2S mRNA EXPRESSION. H.H. Yeh*, E.V. Grigorenko and T. Y. Rikhter. Dept. Physiology & Pharmacology, Bowman Gray Sch. Med., Winston-Salem, NC 27157.

The modulation of the GABA$_A$ receptor by ethanol may be dependent on GABA$_A$ receptor subunit composition. Since the expression of many GABA$_A$ receptor subunits are developmentally regulated, such dynamics should reveal to advantage as to whether ethanol sensitivity is related to GABA$_A$ receptor subunit make-up. Here, we combined patch clamp recording and RT-PCR in single immature Purkinje cells to link ethanol-GABA interaction with GABA$_A$ receptor subunit mRNA expression, focusing on the long and short splice variants of the γ2 subunit (γ2L &γ2S).

Purkinje cells acutely dissociated from postnatal day(PD)-1, -3, -5, -7, -9 and -11 rat cerebella, were chosen for study. At each age examined, GABA-activated whole-cell current responses were potentiated upon co-application of 50 mM ethanol. Profiling of GABA$_A$ receptor subunit mRNAs in these cells revealed the expression of α, β and γ subunit mRNAs. The γ subunit expressed prior to PD-7 was that of γ2S. The expression of γ2L was not evident in individual Purkinje cells until PD-7. In whole cerebellar tissue, γ2S mRNA expression was found throughout postnatal development but that of γ2L mRNA only beyond PD-5. Other subunit messages also appear developmentally regulated.

Our results indicate that ethanol modulation of GABA$_A$ receptor function in cerebellar Purkinje cells is present prior to the emergence of, and thus independent of, γ2L mRNA expression and that the dynamics of neuroreceptor expression in development can provide insights into the specificity of ethanol-neuroreceptor interactions.

824.7

PRENATAL ETHANOL EXPOSURE ALTERS MODULATION OF THE $GABA_A$ RECEPTOR CHLORIDE CHANNEL COMPLEX IN ADULT OFFSPRING. D.D. Savage, L.L. Paxton, H. Wu, and A.M. Allan* Dept. of Pharmacology, Univ. of New Mexico H.S.C., Albuquerque, NM 87131-5316

The amino acid neurotransmitters glutamate and GABA have been implicated as neurochemical correlates underlying the electrophysiological and behavioral deficits observed in Fetal Alcohol Syndrome. To further investigate prenatal ethanol-induced changes in the GABA system, we measured the ability of GABA and four modulatory agents that affect $GABA_A$ receptor-stimulated ^{36}chloride ion flux in membrane vesicles prepared from hippocampus, cerebellum and frontal cortex of adult offspring from *ad lib* chow, pair-fed and ethanol fed rat dams. Prenatal ethanol treatment involved the consumption of a liquid BioServ diet containing 5% (v/v) ethanol by rat dams throughout gestation.

Dose dependent GABA-stimulation of chloride flux was not significantly affected by the prenatal ethanol exposure in any of the brain areas examined. In the frontal cortex, prenatal ethanol exposure significantly attenuated both the positive modulatory effects of flunitrazepam (FLU; 25 μM) and alphaxalone (ALPH; 25 μM) as well as the negative modulation by the inhibitory neurosteroid, 5 α-pregnan-3 β -ol- 20-one sulfate (PS; 25 μM) and the benzodiazepine inverse agonist FG-7142 (FG; 10 μM) on GABA-stimulated $^{36}Cl^-$ flux. In the cerebellum, prenatal ethanol exposure diminished the modulatory influences of the benzodiazepines but not the neurosteroids. In hippocampus, prenatal ethanol exposure enhanced the effect of positive modulators (FLU and ALPH) but diminished the effects of negative modulators (FG and PS). These findings suggest that prenatal ethanol exposure produces long-lasting effects on the modulatory sites of the $GABA_A$ receptor complex. Further studies will be required to determine whether these alterations are a compensatory mechanism in response to the early ethanol insult or if these alterations contribute to the electrophysiological and behavioral deficits observed in fetal ethanol exposed offspring.

824.8

EFFECT OF PRENATAL ALCOHOL EXPOSURE ON SEROTONIN TRANSPORTER BINDING SITES IN RAT BRAIN. H. M. Zafar , S. Shelat, E. Redei and S. M. Tejani-Butt*. Depts. of Psychiatry and Pharmacology, Univ. of Penn. Sch. of Med., Phila., PA 19104.

Fetal alcohol exposure (FAE) is known to have long-term effects on behavior which include hyperactivity and altered stress response. FAE has also been reported to decrease serotonin (5-HT) levels, turnover, release and uptake, as well as alter 5-HT receptor sites in several brain regions of offsprings. Since presynaptically located 5-HT transporter (5-HTT) sites serve as markers of 5-HT innervation, the present study examined the effects of FAE on 5-HTT binding sites in several discrete brain regions of the developing male and female offspring. Time-pregnant dams were fed liquid ethanol diet, isocaloric diet without ethanol or normal rat chow. Male and female offspring were sacrificed at 21, 40 and 60 days of age. The brains were removed and sectioned for autoradiographic analysis of ^{3}H-cyanoimipramine (^{3}H-CNIMI) binding to 5-HTT sites. The results indicate that FAE had a significant time and sex dependent effect on 5-HTT sites. In FAE-females, 5-HTT sites were decreased in the amygdala and hypothalamus and increased in the hippocampus and dorsal raphe nucleus as early as day 21. However, 5-HTT sites increased in the amygdala in day 40 FAE-females, and this increase persisted in the amygdala and hippocampus in adulthood. In contrast, 5-HTT sites were found to be unaltered in immature FAE-males, but decreased in the amygdala and hypothalamus and increased in the hippocampus and dorsal raphe nucleus in adult FAE-males; a pattern similar to immature FAE-females. (Research funds from USPHS grant NS 31699 and AA 07389).

824.9

EFFECT OF PRENATAL ETHANOL EXPOSURE ON POSTNATAL EXPRESSION OF GLUTAMATE RECEPTORS AND PHYSIOLOGICAL RESPONSES IN BARREL FIELD CORTEX. V. Rema*, W. Huang and F. F. Ebner. Institute for Developmental Neuroscience, Vanderbilt University, Kennedy Center, Nashville, TN 37203.

Prenatal ethanol exposure leads to alterations in the expression of glutamate receptors in postnatal rat cortex. There is reduced expression of NMDA receptor subunits NR1, NR2A/B as detected by immunocytochemical analysis, which is maintained for at least 3 months after birth. However we do not see a similar reduction in the expression of AMPA receptor subunits at the ages examined. Compared to normal animals the cortex of rats prenatally exposed to ethanol showed an increase in GluR1 immunostaining at P21 and P30, whereas GluR2/3 immunoreactivity was lower at P21 and higher by P30 compared to normal. The expression of GluR4 does not seem to be effected by prenatal ethanol exposure at P21 and P30. We are currently examining the effect of these subunit changes on physiologically recorded plasticity. Extracellular single unit recording suggests that neuronal responses in the primary somatosensory cortex of adult rats are altered following exposure to alcohol during gestation. The main differences compared to normal animal are in the level of spontaneous activity, the amplitude of evoked responses in the center and surround receptive fields and the response latencies. (Supported by NS 13031 and HD 1052)

824.10

CORPUS STRIATUM HISTOLOGICAL ALTERATIONS IN ADULT MICE PRENATALLY TREATED WITH DIAZEPAM A. Márquez-Orozco*, M.C. Márquez-Orozco, M.V. Gazca-Ramírez and G. de la Fuente-Juárez. Embryol Dept. School of Medicine UNAM, POB 70-553 México 04510 D.F. México.

Diazepam accumulates in the fetal human and mice corpus striatum, where it produces a delay in the neuroblastic differentiation. An atypical chromatin distribution and less number of fibers. We investigated if the histological alterations of the fetal corpus striatum occurs in adult mice. Single daily doses (2.7 mg/kg) of diazepam were sc. administrated to CD-1 strain female mice, from gestation day 6 to 17. A control received equivalent volumes of saline solution. The offspring's were wet-nursed by non-treated mice, weaned and kept for 240 days. The motor activity and swimming pattern were periodically measured from the 6th day until the 8th month. Mice were deeply anesthetized, perfused with 10% formaline, and the brains were removed, fixed and stained for myelin, nerve cells, and axons identification; a selected blocks were impregnated with the fast-Golgi technique. Light microscopy demonstrated in diazepam mice atypical distribution of neurons, less number of fibers, and accumulation of glial cells around vessels, and in the bundles fibers. Behavioral analysis of the experimental group (swimming, and spontaneous motor activity) correlated well with the persistent histological alterations.

824.11

PERSISTING EFFECTS OF PRENATAL EXPOSURE TO DIAZEPAM ON SEXUAL BEHAVIOR OF FEMALE MICE. L.A.I. Hernández-Alvarez*, A. Martínez-Vargas, B. Victoria-Romero, A. Márquez-Orozco and M.C. Márquez-Orozco. Unit for Research in reproduction. School of Medicine, BUAP and Dept. of Embryology. School of Medicine, UNAM, México 04510 D.F., México.

We have shown in forgoing works, effects of prenatal exposure to diazepam (DZP) on sexually dimorphic reproductive behaviors. In this work, we assessed the sexual behavior of senile females CD-1 strain mice exposed to DZP during gestation. One group of female mice was s.c. treated with DZP (2.5 mg/kg/d) from the 6th to the 17th days of gestation and a control group received saline sol. On the 27th month of age, the spontaneous female sexual activity to males from the same breeds was tested and videorecorded under red light. Precopulating and copulating activities were evaluated. No difference was found in precopulating behaviors from both groups, but during copulating stage lordotic indexes and proportion of lordotic females were greater in experimental animals. Results indicate persistent and long-lasting mating facility patterns of prenatal exposure of DZP on sexual behaviors which could be due to permanent modification of neurotransmission by the drug during development.

824.12

HISTOLOGICAL CHANGES IN THE RETINA OF MOUSE FETUSES EXPOSURE TO DIAZEPAM. M.C. Márquez-Orozco*, A. Márquez-Orozco, M.V. Gazca-Ramírez and G. de la Fuente-Juárez. Embryol. Dept. School of Medicine UNAM, POB 70-553 México 04510 D.F. México.

We investigated if diazepam (DZ) 1.0 mg/kg dose produces similar histological effects in the fetal retina as with 2.7 mg/kg dose. Three gestating CD-1 strain mice groups were injected daily sc. from day 6 to 17, the first group with single daily DZ doses (2.7 mg/kg), the second group with single daily DZ, doses (1.0 mg/kg) the third group received saline solution (S). A fourth group was non-treated (NT). All were killed with CO_2 atmosphere the 18th. day, and the fetus removed. Their eyes were fixed with 2.5% glutaraldehide, post-fixed in OsO_4 and embedded in epoxy resin. The semifine sections were stained with toluidine blue and observed under the light microscope. The fetal retina in both DZ groups revealed in the neuroblastic layers delay cellular differentiation. A greater nuclear density of the retinal cells were observed. The cells showing nuclei with clumps of heterochromatin atypically distributed. S and NT groups exhibited neither histological differences in the retina and the cells showing nuclei with normal heterochromatin typically distributed. Results give evidence that both DZ doses produces histological changes in the fetal retina.

824.13

EFFECTS OF FLUMAZENIL BENZODIAZEPINE DEPENDENCE IN MAN. S. W. Woods*, M. I. Rosen, L. H. Price, H. R. Pearsall, F. A. Hameedi, D. W. Gallager, D.S. Charney, G. R. Heninger, T.R. Kosten. Yale University, 34 Park Street, New Haven, CT 06519

Preclinical studies suggest that the benzodiazepine (BZ) receptor antagonist flumazenil (FLU) in single doses can reduce established BZ dependence. The goal of this ongoing study is to determine whether FLU has this effect in BZ dependent human subjects (Ss). Barbiturate pretreatment is employed to attenuate FLU- induced acute withdrawal. Method: Thus far, 10 methadone maintained polysubstance abuse inpatient Ss have been randomly assigned to FLU (n=5) or placebo (PLA, n=5) groups. FLU Ss were dependent on BZs for 3.0±0.7 yrs and were stabilized for 18±7 d on 5.6±2.2 mg/d alprazolam (ALP) equivalent. After stabilization, a single dose of phenobarbital (PBS) at 30 times (x, n=3) or 60x (n=2) the ALP daily stabilization dose was administered p.o. followed after 2.5 hrs by FLU 1.5 mg i.v. per S over 15 min. PLA Ss dependent on BZs for 6.8±6.0 yrs were stabilized for 18±6 d on 6.0±1.9 mg/d ALP equivalent. These Ss also received PBS at 30x (n=3) or 60x (n=2) the ALP dose, but PBS was followed by double-blind placebo (PLA) i.v. over 15 min. One hr after FLU/PLA, BZ was abruptly discontinued via single blind placebo substitution in both groups. Abstinence symptoms were rated using the Ribicoff Abstinence Rating Scale (RARS) for the next ten days. Results: Results to date indicate that FLU was well tolerated in these BZ dependent Ss when given after PBS. RARS scores after BZ discontinuation peaked as expected at 2-3 days in both groups but are approximately 50% lower in the FLU-treated Ss. "Escape" medication doses were lower for FLU (0.6±0.9 mg ALP equivalent/10d) than for PLA (3.2±3.1 mg) Ss. Discussion: These results suggest that FLU in a single dose when administered with PBS may be able safely to reduce established BZ dependence in man. Further research is needed to investigate dose-response relationships.

824.14

THE EFFECT OF INTRA-AMYGDALOID ADMINISTRATION OF FLUMAZENIL IN DIAZEPAM-DEPENDENT RATS. J.W. SLOAN*, E.P. Wala, X. Jing. Dept. of Anesthesiology, College of Medicine, Univ. of KY., Lexington, KY 40536

The amygdala is an important brain site for the anxiolitic and anticonvulsant actions of benzodiazepines (BZS). Autoradiographic studies indicate a high density of BZ receptors (BZR) (predominantly type-2) in the basolateral amygdaloid nuclear complex. The aims of the present study were to test the ability of the BZR antagonist, flumazenil (FLU), to precipitate an abstinence syndrome in the ventromedial lateral (LaVM) and basolateral (BLA) amygdaloid nuclei in diazepam (DZ)-dependent rats. The rats were exposed for 3 wks to DZ (540 mg/wk) slowly released from subcutaneously implanted silastic capsules. FLU (25 ug) and DMSO vehicle were microinjected into LaVM (AP=5.7; RL=4.8; V=2) (n=6) and BLA (AP=5.7; RL=4.8; V=1)(n=5). The abstinence signs, behavioral activities and EEG were recorded for 10 min before and 40 min after microinjections. The Precipitated Abstinence Score (PAS), Behavioral Score (BS) and the changes in Total Power (TP) of the EEG recorded at the sites of microinjections were determined. FLU did not produce either a significant PAS or a significant BS in BLA and LaVM. FLU evoked clonic convulsions (1 rat) and twitches and jerks (P<.05) in BLA. No signs of abstinence were precipitated by FLU in LaVM. FLU tended to increase TP of low and high frequency bands in BLA but not in LaVM. DMSO did not alter TP in BLA and LaVM. The present data indicate that except for convulsive signs a surprisingly mild abstinence was evoked by FLU in both nuclei of lateral amygdala in rats dependent on DZ. (supported by NIDA grant DA02195)

824.15

INTRATHECAL (IT) ADMINISTRATION OF FLUMAZENIL AND PK11195 PRODUCE AN ABSTINENCE SYNDROME IN DIAZEPAM-DEPENDENT RATS. E.P. Wala,* J. W. Sloan, X. Jing, P.H. Holtman, Dept. of Anesthesiology, College of Medicine, Univ. of KY., Lexington, KY 40536.

The actions of central and peripheral benzodiazepine receptor (BZR) antagonists, flumazenil (FLU) and PK11195 (PK), at the spinal level were determined in rats subcutaneously implanted for 3 wks with silastic capsules filled with diazepam (DZ) (540 mg/wk). Control rats were exposed to empty capsules. The rats were implanted with EEG electrodes into the ventral thalamic nucleus (Th), CA1 area of hippocampus (H) and parietal (PC), frontal (FC) and cerebellar (CbC) cortices and with an intrathecal (IT) catheter into the spinal subarachnoid space (vicinity of T-12). After recovery, FLU (0.1 mg/kg), PK (0.05 mg/kg) and vehicle were injected (10ul) IT. The EEG, abstinence signs and behavioral activities were recorded. The Precipitated Abstinence Score (PAS), Behavioral Score (BS) and changes in Total Power (TP) for slow (4-12Hz) and fast (18-26Hz) frequency bands were determined. Intrathecal FLU and PK precipitated a significant PAS but not a significant BS in DZ dependent rats. Intrathecal PK decreased TP of fast waves in Th,H,PC and CbC but not in FC. FLU did not alter TP. In control rats PAS, BS and changes in TP were not significant. Thus, in the spinal cord both central (predominantly type-2) and peripheral BZR are involved in dependence on DZ as indicated by the PAS produced by FLU and PK. The lack of effect of IT FLU on EEG may be related to the low density of type-1 BZR in the spinal cord. (NIDA grant DA02195).

824.16

CONTINGENT TOLERANCE, BUT NOT WITHDRAWAL SYMPTOMS, IS MAINTAINED FOR 14 DAYS WITHOUT TREATMENT FOLLOWING DIAZEPAM EXPOSURE IN KINDLED RATS. Kippin, T.E.[1], St. Denis, M.[2], Kalynchuk, L.E.[2], & Pinel, J.P.J.[2] 1. Centre for Studies in Behavioral Neurobiology, Concordia Univ., Montreal, Que; 2. Dept of Psych, Univ. British Columbia, Vancouver, B.C.

Tolerance to a drug's effect and symptoms of withdrawal are often considered to be different manifestations of the same underlying mechanisms, however, few adequate attempts to dissociate these phenomena have been made. In the present study, amygdala-kindled adult male Long-Evans rats were used to examine tolerance to the anticonvulsant effects of and symptoms associated with withdrawal from diazepam (2.5 mg/kg). Following kindling, rats were divided into three groups. On each of 10 treatment trials, rats received either diazepam 1 hr before (drug-before group) or after (drug-after group) a convulsive stimulation or vehicle 1 hr before or after convulsive stimulation (vehicle-control group). Then, all rats received convulsive stimulations 1 hr following diazepam (tolerance test) and 1 hr following vehicle (stimulation tests). Consistent with previous reports, tolerance to diazepam's anticonvulsant effects was contingent upon receiving a convulsive stimulation during drug exposure--only drug-before rats were tolerant. However, both the drug-before and drug-after groups displayed significant withdrawal effects as demonstrated by increased duration and severity of convulsions on stimulation tests--larger withdrawal effects were displayed by the drug-before rats than the drug-after rats. Following a 14-day retention interval (without drug or stimulation), all rats received a second series of tolerance and stimulation tests. As previously reported, contingent tolerance was still present in drug-before rats. However, neither the drug-before nor the drug-after rats displayed any evidence of withdrawal effects. The finding that contingent tolerance, but not withdrawal effects, was maintained over the retention interval suggests that different mechanisms underlie these phenomena.

824.17

EFFECTS OF DIAZEPAM ON $GABA_A$ RECEPTOR FUNCTION IN THE TOLERANT AND WITHDRAWAL RATS. S. Toki, T. Saito*, A. Nabeshima, H. Ozawa, S. Hatta[1], M. Watanabe, and N. Takahata Department of Neuropsychiatry and Pharmacology[1], School of Medicine, Sapporo Medical University, Sapporo, 060 Japan

Alterations in $GABA_A$ receptor function were studied in diazepam (DZP)-dependent rats. Physical dependence on DZP was induced in male Fischer rats by the drug-admixed food (DAF) method. Using the DAF method, severe physical dependence on DZP is characterzed by spontaneous convulsions during DZP withdrawal developed. In comparison with control rats, 10μM GABA-dependent $^{36}Cl^-$ influx in withdrawal rats wassignificantly increaced, whereas there was no significant difference between the tolerant and control rats. While enhancement of GABA-dependent $^{36}Cl^-$ influx by the addition of EtOH and flunitrazepam (FZ) was recognized in the control, there was no such effect of EtOH or FZ in the DZP-tolerant animals. On the other hand, GABA-dependent $^{36}Cl^-$ influx was enhanced by FZ in the withdrawn group. In a [^{3}H] FZ binding assay to benzodiazepine (BZ) receptors, Bmax values were significantly increased in DZP-withdrawn animals, but decreased in the DZP-tolerant group as compared with the control. When [^{3}H] muscimol binding was examined, the Kd of high affinity sites of the $GABA_A$ receptor in withdrawn rats was significantly lower than in the control. In low-affinity binding sites, the values of Kd and Bmax were significantly decreased as compared with those in the control. The present study indicates that GABAergic transmission involving the regulation of GABA-dependent chloride channels is altered in DZP-dependent rats.

824.18

GENDER-SPECIFIC CHANGES IN CRF CONTENT AND CORTICOSTERONE LEVELS FOLLOWING CHRONIC BENZODIAZEPINE EXPOSURE IN RATS. M. A. Wilson* and R. Biscardi. Department of Pharmacology, Univ. South Carolina School of Medicine, Columbia, SC 29208.

Acute stress induces sex-specific changes in GABA/benzodiazepine (BZ) receptors and corticosterone release in rats. Gender-related differences have also been observed in GABA/BZ responses after chronic benzodiazepine exposure. The present study compared changes in CRF content and corticosterone release following benzodiazepine exposure in ovariectomized female (OVX) and sham-operated male rats. After treatment with vehicle or diazepam-filled (DZ) silastic capsules for 3 days (acute) or 3 weeks (chronic), CRF content in punches from eight brain areas and serum corticosterone were determined in groups of unstressed (habituated to sacrifice procedures) and swim-stressed (10 min prior to sacrifice) rats. In OVX females, acute and chronic DZ exposure attenuated the stress-induced increase in corticosterone by 31% and 32%, respectively ($F=13$, $p<0.002$ for DZ effect). Males showed nonsignificant decreases in corticosterone levels (15%) after acute and chronic DZ exposure. Chronic diazepam exposure significantly increased CRF content in amygdala of unstressed OVX females, but not males ($F=8$, $p=0.01$ for sex-DZ interaction). Median eminence levels of CRF were reduced by chronic DZ treatment in unstressed males, but not in OVX females ($F=6.5$,$p=0.02$ for interaction). Several brain areas (preoptic area, locus coeruleus, paraventricular nucleus) showed male-OVX differences in CRF levels, but no effect of DZ exposure. CRF content in some areas was not altered by gender or DZ treatments. The results suggest BZ agonists attenuate stress-induced corticosterone release to a greater degree in females than males, but that tolerance does not develop to this effect. Chronic benzodiazepine exposure appears to modulate CRF content of certain brain areas in a gender-specific manner. (Support: DA05932 to MAW).

824.19

DRUG DISCRIMINATION ANALYSIS OF PARTIAL AGONISTS AT THE BENZODIAZEPINE (BZ) MODULATORY SITE. N. A. Ator*, M. A. Kautz, C. A. Sannerud[1], and R. R. Griffiths. Johns Hopkins Univ. Sch. Med. and [1]Natl. Inst. on Drug Abuse, Balt., MD 21224.

The discriminative stimulus effects of partial agonists at the Bz site were studied in rats and baboons trained to discriminate Bz or pentobarbital. In a two-lever drug versus no drug procedure, bretazenil, imidazenil, and U-78875 reliably occasioned drug-appropriate responding in animals trained to discriminate diazepam and pentobarbital but there was less reliable generalization in animals trained to discriminate lorazepam. In rats trained to discriminate two doses of midazolam under a three-lever procedure, bretazenil occasioned 100% drug-appropriate responding only on the lever paired with the 0.32 mg/kg dose but not the 3.2 mg/kg dose. In rats trained to discriminate lorazepam from pentobarbital under a three-lever procedure, imidazenil occasioned responding on both drug-paired levers; although, across the dose range, responding was higher on the lorazepam lever for rats trained to discriminate lorazepam first and pentobarbital second. In interaction studies in rats, U-78875 antagonized the effects of lorazepam, had little effect on diazepam, and potentiated the effects of pentobarbital. There appeared to be a behavioral component to the ability of U-78875 and bretazenil to antagonize lorazepam's effects in baboons: the compound antagonized lorazepam when the baboon did not generalize to it but potentiated lorazepam if the partial agonist shared discriminative effects with lorazepam. Supported in part by NIDA DA04133.

824.20

ALPRAZOLAM DEPENDENCE PREVENTED BY SUBSTITUTING WITH THE ß-CARBOLINE ABECARNIL G. Pinna, R. Galici, H.H. Schneider, D.N. Stephens* and L. Turski. Research Laboratories of Schering AG, D-13342 Berlin, Germany.

Abrupt termination of the treatment of humans with benzodiazepines (BDZs) leads to a rapid-onset discontinuation syndrome. For this reason, there is considerable interest in discovering means of weaning patients away from BDZs without the risk of discontinuation syndrome. We have employed methods for electroencephalographic (EEG) monitoring of seizures, for electromyographic monitoring of muscle tone, and for detecting anxiety-like behavioral changes after discontinuation of long-term treatment with sedative drugs in mice for controlled and standardized assessment of dependence liability of alprazolam. Alprazolam, a BDZ with a short half-life, was chosen because it has replaced diazepam as the most prescribed anxiolytic drug since the end of eighties. Male NMRI mice, 20-24 g, were subjected to s.c. injections of 6 mg/kg of alprazolam or vehicle given twice daily for 12 days. Long-term treatment with alprazolam led to a rapid loss of its depressant action on exploratory activity in non-habituated mice. Monitoring of withdrawal signs started on the day following the last administration of alprazolam or vehicle. The intensity of the discontinuation syndrome (EEG seizures, rigidity, anxiety) increased slightly over first 2-3 days of withdrawal, the symptoms were most pronounced during the next 7-14 days, and abated slowly up to withdrawal days 21-28. Replacement of alprazolam treatment with the ß-carboline selective agonist abecarnil (6 mg/kg/d for 7 d) prevented the occurrence of withdrawal signs; when replacement treatment with abecarnil was subsequently terminated no signs of dependence were detected. Replacement of alprazolam treatment with the ß-carboline antagonist ZK93426 (ethyl-5-isopropoxy-4-methyl-ß-carboline-3-carboxylate; 20 mg/kg/d for 7 d) did not prevent the discontinuation syndrome. Replacement therapy with abecarnil after long-term treatment with the BDZs offers novel method for rapid tapering. The use of the BDZ antagonists for tapering long-term treatment with BDZs is not justified by the experimental data.

824.21

NATURALLY OCCURRING BENZODIAZEPINE-LIKE COMPOUNDS IN FOOD AND PLANTS: THEIR IMPLICATION IN HEALTH AND DISEASES. R. Avallone, L. Corsi, D. Braghiroli, C. Parenti, M. Di Bella,, I Venturini°, M.L. Zeneroli° and M. Baraldi*. Depts of Pharmaceutical Sciences and of Internal Medicine°, Modena University, Italy.

Since the identification of the Benzodiazepine (Bz) receptors many studies have been performed to search for natural Bz receptor ligands for these recognition sites. Many substances have been found to act either specifically or nonspecifically at Bz receptors. Recently, however, some investigators have described the presence of Diazepam, Nordiazepam and other halogenated Bz in human and animal brain tissues. Several authors gathered further chemical evidence of the presence of Bz and metabolites in natural sources like serum and milk. Since synthetic pathway for the production of halogenated Bz are not yet found in animal tissues, it is likely to surmize that these Bz are of exogenous source. The presence of halogenated Bz have been described in plants and in food. It must be stressed however that there are also other Bz-like compounds, and among them the so called "endozepines" which are nonhalogenated substances, which are still poorly characterized. Herein we report that using extraction protocol, HPLC purification, receptor binding displacement and selective antibenzodiazepine antibody we identified several Bz-like compounds in foods such as carobs and potatoes and in medicinal plants such as Matricaria Chamomilla with mean concentrations of 0.5 - 2 ng/g depending on the analyzed part of the vegetable. The presence of Bz-like compounds detected in plants, food stuffs and milk seems to support the hypothesis that exogenously supplied Bz-like compounds could be regarded as exogenous agents which when introduced through the alimentary chain subserve the function to basically activate the Bz receptors for their physiological function. There are however pathological conditions where Bz compounds have been found to accumulate because of the altered metabolism by the liver such liver cirrhosis and fulminant hepatitis. In these pathological conditions we know that caution must be taken to avoid the administration of commercial Bz due to an increased GABAergic tone. The data presented indicate also that the administration of food containing natural Bz must be avoided in order to prevent the occurrance of episodes of encephalopathy in patients with severe liver diseases.The rise of Bz in plasma and in brain might play a role as precipitating factor of hepatic encephalopathy.

DRUGS OF ABUSE: ALCOHOL VI

825.1

CHARACTERIZATION OF CHLORIDE EFFLUX FROM GT1-7 NEURONS: LACK OF EFFECT OF ETHANOL ON $GABA_A$ RESPONSE AT 4° C. M.A. Javors*, T.S. King, X. Chang, C. Levinson, and M.K. Ticku. Departments of Psychiatry, Pharmacology, Physiology, and C&SB, University of Texas HSC, San Antonio, TX 78284.

GT1-7 hypothalamic neurons, which synthesize and secrete GnRH (gonadotrophin releasing hormone), were immortalized from transgenic mice and have been shown to express functional $GABA_A$ receptors. Our previous experiments showed that stimulation of these receptors with muscimol produced an increase of cytosolic [Ca^{2+}] and the release of GnRH. Our previous experiments also showed that ethanol enhanced $GABA_A$-mediated increase in [Ca^{2+}] despite the fact that GT1-7 neurons do not express a γ subunit. GT1-7 neurons were grown to confluence in 100 mm diameter culture dishes and loaded with ^{36}chloride overnight. The next morning cells were washed extensively. The extracellular medium was then sampled to determine chloride efflux. Our results show that chloride efflux increased with increased temperature (tested at 4°, 23°, and 37° C; 4° C was used for the rest of our experiments to minimize background). DIDS, an inhibitor of anion exchange, reduced chloride efflux by 65% with an IC50 of 100 µM. Bumetanide, an inhibitor of Na:K:2Cl cotransport, had no effect on chloride transport at concentrations up to 200 µM. Muscimol, a $GABA_A$ agonist, stimulated chloride efflux with an IC50 equal to 0.5 µM. Bicuculline, a $GABA_A$ antagonist, reversed the muscimol effect with an IC50 of 13 µM. Ethanol (46 mM) had no effect on muscimol-induced chloride efflux at 0.5, 1.0, and 10 µM muscimol. Our results suggest that stimulation of $GABA_A$ receptors causes an efflux of chloride from GT1-7 neurons. The lack of an effect of ethanol under these conditions (4° C) is consistent with the current hypothesis that a γ subunit may be necessary for the effects of low concentrations of ethanol at $GABA_A$ receptors. (Supported by NIAAA grant AA10112.)

825.2

EVALUATION OF THE DISCRIMINATIVE STIMULUS EFFECTS OF GABAERGIC DRUGS IN MUSCIMOL-TRAINED RATS. Hendree E. Jones and Robert L. Balster*. Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298-0310.

The discriminative stimulus effects of GABAergic drugs were evaluated in rats trained to discriminate the direct $GABA_A$ agonist, muscimol (1.0 mg/kg I.P.), from saline under a two-lever fixed ratio (FR) 32 schedule of food reinforcement. Another direct $GABA_A$ agonist, THIP, produced full substitution for muscimol. Diazepam, an allosteric modulator of GABA-mediated postsynaptic inhibition, yielded a maximum of 54% muscimol-lever responding at a dose which decreased rates of responding. Partial substitution for muscimol (maximal levels of 71% muscimol-lever responding) was also produced by the GABA agonist progabide. The GABA uptake inhibitor, tiagabine, produced no greater than 48% muscimol-lever responding. Valproic acid, a reversible GABA transaminase inhibitor, failed to substitute for muscimol and vigabatrin, an irreversible GABA transaminase inhibitor, yielded a maximal 46% muscimol-lever responding. These results demonstrate the pharmacological specificity of muscimol discrimination by showing that only direct agonists for the GABA site on the $GABA_A$ receptor complex produce full substitution. GABA agonists acting by other mechanisms can be distinguished from muscimol and THIP in this procedure. (Research supported by NIDA grants DA-01442 and DA-05665).

825.3

CHARACTERIZATION OF GLUTAMIC ACID DECARBOXYLASE PROTEIN FUNCTION AND mRNA CONTENT IN GENETIC MODELS OF SEVERE OR MILD WITHDRAWAL NEUROEXCITABILITY. K.J. Buck*, T. Lischka and D. Wu. Department of Medical Psychology, Oregon Health Sciences University, Portland, OR 97201-3098, USA.

Physical dependence and withdrawal from ethanol and other central depressants shows the influence of heritable factors, but none of the genes responsible has been identified. By association and interval mapping, we identified a quantitative trait locus (QTL) which accounts for more than 40% of the genetic variance in ethanol withdrawal neuroexcitability in C57BL/6J (B6) X DBA/2J (D2) recombinant inbred (BXD RI) strains. Analyses using a B6D2F2 intercross confirmed that a QTL affecting withdrawal severity is linked to the microsatellite marker *D2Mit9*, which localizes to the same region of chromosome 2 as *Gad-1* (37-43 cM). *Gad-1* encodes glutamic acid decarboxylase (GAD), which catalyzes the rate limiting step in the synthesis of the major known inhibitory neurotransmitter γ-aminobutyric acid (GABA). Numerous studies have implicated the synaptic actions of GABA in neuroadaptation to ethanol associated with physical dependence and withdrawal.

DBA/2J mice are a well characterized genetic animal model expressing severe ethanol dependence and withdrawal neuroexcitability, whereas B6 have mild withdrawal reactions. We found that D2 mice demonstrate lower GAD enzyme activity as compared to B6 mice. Northern blot analyses using RNA isolated from ethanol-naive and ethanol-dependent B6 and D2 mice show that GAD mRNA content (3.7 kb and 5.7 kb isoforms, encoded by *Gad-1* and *Gad-2*, respectively) is regulated by chronic ethanol treatment. These results indicate that genetic differences in ethanol withdrawal neuroexcitability may be mediated, in part, by differences in GAD expression and function. (Supported by NIH grants RO1 AA06243, PO1 AA08621, and T32AA07468)

825.4

ETHANOL INCREASES $GABA_A$ RECEPTOR-MEDIATED CURRENTS IN CEREBELLAR PURKINJE NEURONS IN RAT BRAIN SLICES. W.R. Proctor* and T.V. Dunwiddie, Dept. Pharmacol., Univ. Colo. Hlth. Sci. Ctr. and Vet. Adm. Med. Ctr., Denver, CO

This study was designed to determine if GABAergic responses evoked in cerebellar Purkinje cells are modulated by ethanol, and to further examine the cellular mechanisms that might be involved. Brain slices were prepared from the cerebellar vermis, maintained under *in vitro* conditions, and Purkinje neurons were readily visualized using DIC optics. The slices were stored and recorded at room temperature, drugs were added to the superfusing aCSF, and patch electrodes containing K^+ or Cs^+- gluconate were used while recording in current-clamp or voltage-clamp modes. Stimulation of the parallel fibers in the molecular layer produced either epsp, ipsp or epsp/ipsp responses. The ipsps were rapidly and reversibly blocked by 10 μM bicuculline and were usually enhanced (10-30%) by superfusion of 80 mM ethanol. Epsps and membrane resistance did not appear to be significantly altered, whereas the spontaneous firing rate was typically depressed by ethanol. In addition, the local pressure application of GABA (10 μM) to the proximal dendritic region resulted in outward current responses that were greatly depressed by bicuculline but were not significantly affected by ethanol, whether short (5-10 msec) or long (1 sec) pressure applications were used. Pretreatment of the slice with the ß-adrenergic agonist, isoproterenol (500 nM), did not seem to enhance the effects of ethanol. The finding that ethanol enhances bicuculline-sensitive ipsp responses in Purkinje neurons under these *in vitro* conditions, suggests that $GABA_A$ receptor-mediated events are involved in ethanol action in the cerebellum.

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825.5

CHRONIC ETHANOL EXPOSURE AND WITHDRAWAL SELECTIVELY INCREASE DIAZEPAM-INSENSITIVE [^{3}H]RO 15-4513 BINDING IN MOUSE CEREBELLUM. H.C. Becker* and M.F. Jarvis, Med. Univ. of South Carolina and VAMC, Charleston, SC 29425 and Rhône-Poulenc Rorer Central Research, Collegeville, PA.

The partial benzodiazepine inverse agonist, RO 15-4513 has been shown to block some behavioral and biochemical effects of ethanol (EtOH). [^{3}H]RO 15-4513 labels two classes of recognition sites in the mammalian cerebellum, a diazepam-sensitive (DZ-S) site and a diazepam-insensitive (DZ-IS) site that may represent a novel subtype of the GABAa receptor. The present studies were conducted to examine the effects of chronic EtOH exposure on both total and DZ-IS [^{3}H]RO 15-4513 binding in cerebellum from C3H mice. Mice received either 64 hr EtOH intoxication via inhalation chambers (BEC = 165-185 mg/dl) or no EtOH exposure. Mice were sacrificed at the end of the EtOH exposure (HR 0) or 8 hours post-withdrawal (HR 8). Ligand saturation studies (0.3-50 nM) were conducted on pooled cerebellar tissue from 3-4 mice. In control mice, the binding parameters for [^{3}H]RO 15-4513 were Kd = 3.9 ± 1.1 nM, Bmax = 3220 ± 300 fmol/mg protein. The binding parameters for [^{3}H]RO 15-4513 in the presence of 10 μM DZ (DZ-IS) were Kd = 3.0 ± 0.4 nM, Bmax = 953 ± 108 fmol/mg protein. Neither EtOH intoxication (HR 0) nor EtOH withdrawal (HR 8) significantly altered total [^{3}H]RO 15-4513 binding in the mouse cerebellum. However, DZ-IS [^{3}H]RO 15-4513 binding density was significantly increased (60%) by EtOH intoxication and by (75%) EtOH withdrawal. These results suggest that chronic EtOH exposure and withdrawal selectively increases DZ-IS [^{3}H]RO 15-4513 binding sites in mouse cerebellum. These data agree with other studies indicating an increase in mRNA and protein levels for the α6 subunit, which encodes DZ-IS RO 15-4513 binding at GABAa receptors in cerebellum.

825.6

CHRONIC ETHANOL TREATMENT DECREASES CENTRAL-TYPE BENZODIAZEPINE RECEPTORS IN RAT BRAIN. X. Ren, S.C.Pandey, M.R.Piano, J.M.Davis*, G.N.Pandey. Department of Psychiatry, University of Illinois at Chicago, IL 60612.

The benzodiazepine receptor is an allosteric modulatory site present on most gamma-aminobutyric acid (GABA) receptor channels. Several lines of evidence suggest that some of the central effects of alcohol may be mediated by the GABA-benzodiazepine-chloride channel receptor complex. The results of benzodiazepine receptor studies in the brain after chronic ethanol exposure appear to be inconsistent. To further examine the role of central benzodiazepine receptors in alcohol dependence, we studied the effect of 60 days of ethanol treatment on central benzodiazepine receptors in the rat cortex. Male Sprague Dewley rats were fed the Lieber-Decarli liquid diet containing ethanol (9% v/v) or control liquid diet for 60 days. Rats were sacrificed and cortices were separated out for measurement of binding indices of central benzodizepine receptors by binding techniques using [^{3}H]-R015-1788 as ligand. We observed that maximum binding sites (B_{max}) of [^{3}H]-R015-1788 binding (763 ± 75 fmol/mg protein) were significantly lower (26%) in the cortex of ethanol-fed rats compared to B_{max} (1026 ± 104 fmol/mg protein) in the cortex of pair-fed control rats. There were no significant changes in Kd values among the groups. These results suggest that decreased central benzodiazepine receptors may be involved in the process of alcohol dependence.

825.7

THE IMIDAZOBENZODIAZEPINE INVERSE AGONIST RO19-4603 (RO19) ATTENUATES ETHANOL (EtOH) ORAL SELF-ADMINISTRATION (OSA) IN SPRAGUE-DAWLEY RATS. G. G. Blakley*, M. E. Paterson, H. L. June, and M. J. Lewis
Neurobehavioral Laboratory, Dept. of Psychology, Temple University, Philadelphia, PA 19122 and IUPUI, Indianapolis, IN 46202

Benzodiazapine (BDZ) inverse-agonists have been reported to antagonize several of EtOH's actions including its reinforcing properties. RO19 is a potent imidazobenzodiazepine inverse agonist. Although having a half-life of 15-60 min, a single dose of RO19 has been reported to suppress EtOH OSA in alcohol preferring (P) and non-preferring (NP) rats for as long as 32 hrs. The present experiment examined the effect of RO19 upon OSA of EtOH in a limited access paradigm in randomly bred, free-feeding Sprague-Dawley rats. Animals were given daily, 1 hr concurrent access to H_2O and gradually increasing EtOH concentrations (1%-10%) (v/v). After stable preference of a 10% solution was established, animals were pretreated with i.p. injections of 0.00g/kg, 0.025g/kg, 0.075g/kg, and 0.150g/kg of RO19. RO19 showed a dose-dependent attenuation of EtOH consumption over several hours. These data are consistent with previous research on RO19 in selected rats and suggest that brief occupation of $GABA_A$/BDZ receptor sites by such compounds may alter EtOH reinforcement. (Supported in part by AA06263 and RR08016 and Temple University.)

825.8

ETHANOL SUBSTITUTES FULLY FOR A DIAZEPAM-KETAMINE MIXTURE.

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When ethanol (EtOH) is trained as a discriminative stimulus, drugs that enhance GABAergic neurotransmission (e.g., diazepam, DZP), and drugs that uncompetitively antagonize glutamate neurotransmission at NMDA receptors (e.g., ketamine, KET), substitute for EtOH. However, when drugs from either of these classes are trained as discriminative stimuli, EtOH fails to substitute fully for either type of drug. The present study tested the hypothesis that in rats trained to discriminate a mixture of DZP (5.6 mg/kg) and KET (10 mg/kg) from saline, EtOH would substitute fully for this training mixture. The mixture was trained using a two-lever choice procedure in which food served as a reinforcer under a fixed-ratio 10 schedule. After the discrimination was acquired, dose-effect testing showed full substitution with: the mixture, DZP alone, KET alone, pentobarbital, chlordiazepoxide, dizocilpine and EtOH. These data support the hypothesis that simultaneous activation of GABAergic neurotransmission and blockade of glutamate neurotransmission are critical in producing an EtOH life stimulus. Supported by R01 AA9378.

825.9

SELECTIVE DRUG ANTAGONISM BY LOW LEVEL HYPERBARIC EXPOSURE SUGGESTS A NEW HYPOTHESIS FOR ETHANOL'S INITIAL SITES OF ACTION: ALLOSTERIC MODULATORY PATHWAYS IN LIGAND GATED ION CHANNELS. R.L. Alkana*, D.L. Davies, M.B. Bolger and R.D. Brinton. Alcohol and Brain Research Lab., Department of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Exposure to low level hyperbaric (≤ 12 times normal atmospheric pressure–ATA) helium-oxygen gas (heliox) antagonizes a broad spectrum of ethanol's acute and chronic behavioral effects. Considerable evidence indicates that the antagonistic mechanism is direct and meets predictions based on a competitive pharmacological model. Our recent *in vivo and in vitro* work found that pressure antagonism is selective: exposure to 12 ATA antagonized the effects of drugs which act through allosteric modulation of $GABA_A$ receptors (ethanol, n-propanol), but did not antagonize the effects of drugs that act via high affinity binding as direct agonists or channel blockers (e.g., GABA, picrotoxin and morphine). This work also demonstrated that pressure antagonized allosteric modulatory drugs (diazepam and pentobarbital) that initiate their allosteric effects by high affinity binding. This selective pattern of pressure antagonism, taken in context with: 1) the similar behavioral and pharmacological profiles for ethanol, diazepam and pentobarbital and 2) the mechanistic link for these drugs to allosterically mediated potentiation of $GABA_A$ receptor function, suggests structural or functional elements that represent likely targets for ethanol's initial actions. These sites are the allosteric modulatory pathways which transduce binding events on ligand gated ion channels. The characteristics that make an allosteric pathway sensitive to ethanol may be unique to specific binding sites within a ligand gated ion channel (e.g., $GABA_A$) or may extend to other ligand gated ion channels sensitive to ethanol (e.g., NMDA and/or $5HT_3$). Our current research is directed toward testing this hypothesis (Supported by NIH, NIAAA research grants AA03972 and AA05234).

825.10

THE EFFECTS OF LOW LEVEL HYPERBARIC EXPOSURE ON 4,5,6,7-TETRAHYDROISOXAZOLO-PYRIDIN-3-OL (THIP)-, PENTOBARBITAL- AND ETHANOL-INDUCED LOSS OF RIGHTING REFLEX DURATION IN MICE. D.L. Davies*, A. Manolescu, N.M. Pecolatto, J.A. McCuin and R.L. Alkana. Alcohol and Brain Research Lab., Department of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Exposure to 12 atmospheres absolute (ATA) helium oxygen gas (heliox) antagonizes behavioral effects of ethanol, n-propanol and diazepam, but not morphine, isoniazid, picrotoxin and *dl*-allylglycine. Recent biochemical studies found that exposure to 12 ATA heliox antagonized ethanol-potentiation of GABA-activated ^{36}Cl uptake in mouse cerebrocortical membrane vesicles (microsacs) without altering GABA-activation *per se*. The pattern of the antagonism indicates that exposure to 12 ATA heliox acts in a selective manner on drugs that act through allosteric modulation. The present study further investigates the selectiveity of low level hyperbaric exposure. C57BL/6J mice were injected ip. with 55 mg/kg THIP (GABA agonist), 60 mg/kg pentobarbital ($GABA_A$ receptor allosteric modulator) or 3.6 g/kg ethanol. When LORR occurred, the mice were placed in cylindrical, stainless steel 18 liter hyperbaric chambers and exposed to either 12 ATA heliox or to control atmospheric conditions of 1 ATA air and/or 1 ATA heliox at ambient temperatures selected to offset drug- and helium-induced hypothermia. Exposure to 12 ATA heliox significantly reduced LORR duration induced by pentobarbital and ethanol but did not significantly reduce LORR duration induced by THIP. These results add to the evidence that low level hyperbaric exposure selectively antagonizes the effects of drugs which act through allosteric modulation of $GABA_A$ receptors (e.g., ethanol, diazepam and pentobarbital) but does not antagonize the effects of drugs that act via high affinity binding as direct agonists or channel blockers (e.g., GABA, picrotoxin and morphine) and suggest that allosteric modulating pathways which transduce binding events on $GABA_A$ and other ligand-gated ion channels may represent initial sites of action for ethanol. (Supported by NIAAA grant AA03972).

825.11

QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF $[^3H]MK801$ BINDING TO NMDA RECEPTORS IN BRAINS OF RODENTS DIFFERENTIALLY SENSITIVE TO ETHANOL. M.J. Velardo* and N.R. Zahniser. Neuroscience Program and Dept. of Pharmacol., Univ. Colo. Health Sci Ctr., Denver, CO 80262.

Initial sensitivity to ethanol has been linked to its action at several classes of brain ligand-gated ion channels. At low concentrations, ethanol inhibits the actions of glutamate at N-methyl-d-aspartate (NMDA) receptors. Therefore, the analepsis observed in response to ethanol may be mediated, at least in part, by NMDA receptors. Long sleep (LS) and short sleep (SS) mice and high alcohol sensitivity (HAS) and low alcohol sensitivity (LAS) rats have been selectively bred to exhibit differential initial sensitivity to the hypnotic effects of ethanol. Using these rodent lines, specific binding of a K_d concentration of $[^3H]MK801$ (10 nM) was determined using quantitative autoradiography. Assays contained saturating concentrations of glutamate, glycine, and spermine in the presence of EDTA. In the mice, specific $[^3H]MK801$ binding in hippocampus was twice that in cerebral cortex, whereas in the rats, binding in the two brain regions was equivalent. Surprisingly, the ethanol-insensitive SS mice showed higher binding (10-20%) in both hippocampus and cortex. No differences were observed between the HAS and LAS rats in either brain region. Our results suggest that differences in NMDA receptors exist in specific regions of LS and SS mouse brain. However, the HAS/LAS rat results weaken the association between the observed differences in $[^3H]MK801$ binding sites and initial ethanol sensitivity. (Supported by AA 03527)

825.12

WITHDRAWAL FROM CHRONIC ETHANOL EXPOSURE INCREASES SENSITIVITY TO N-METHYL-D-ASPARTATE (NMDA) IN RAT NUCLEUS ACCUMBENS NEURONS.
Z. Nie, S.G. Madamba, and G. R. Siggins*. Dept. of Neuropharmacology and Alcohol Research Center, The Scripps Research Institute, La Jolla, CA 92037.

Behavioral studies suggest that the nucleus accumbens (NAcc) is a brain area involved in the rewarding effects of ethanol (EtOH). We recently reported that low EtOH concentrations decreased NMDA-induced currents in NAcc core neurons *in vitro* (Nie et al., JPET 271:1566, 1994). Several lines of evidence suggest that chronic EtOH treatment alters NMDA function. To determine whether such treatment alters NMDA receptor sensitivity in NAcc neurons, we used voltage-clamp recording in a rat brain slice preparation to compare the NMDA receptor sensitivity of NAcc core neurons taken either from rats maintained in ethanol vapor chambers for 3-4 weeks and then withdrawn for 8-12 hours (EtOH treated; mean BALs: 71-138 mg%), or from rats held for the same period in chambers without ethanol vapor (controls). We rapidly superfused NMDA for 3 min, in the presence of 10 μM CNQX, 1 μM tetrodotoxin and 30 μM bicuculline to prevent indirect or non-NMDA responses. We studied 14 cells (mean RMP: -87; mean spike size: 118 mV). There was no apparent difference in spike size or RMP between neurons of control and EtOH treated rats. At holding potentials around -65 mV, 1 μM NMDA superfusion elicited inward currents in only 1 of 6 NAcc neurons from control slices. However, in slices from EtOH treated rats, 1 μM NMDA evoked clear, reversible inward currents (mean: -41 pA) in all 8 neurons studied, and 0.5 μM NMDA evoked -50 pA inward currents in 1 neuron. These data provide new electrophysiological evidence that withdrawal from chronic EtOH exposure in NAcc core neurons increases the responsivity of NMDA receptors, an effect that could underlie the increased behavioral excitability seen in ethanol-withdrawn animals.

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825.13

THE ANTI-CRAVING AGENT ACAMPROSATE ENHANCES NMDA-MEDIATED EPSPs IN RAT NUCLEUS ACCUMBENS NEURONS.
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Acamprosate (calcium acetylhomotaurinate; LIPHA) is a new drug shown in European clinics to prevent relapse in weaned alcoholics. However, the mechanisms underlying this action are unclear. Therefore, we studied the effects of acamprosate in a slice preparation of the nucleus accumbens (NAcc), a brain region thought to play a role in drug reinforcement. Recent studies in our laboratory (Nie et al., JPET, 271: 1566, 1994) demonstrated that ethanol (EtOH) inhibits glutamatergic NMDA- and non-NMDA-mediated synaptic transmission in the NAcc. We used current- and voltage-clamp recordings of NAcc core neurons and isolated locally-evoked NMDA- and non-NMDA glutamatergic EPSP components with 20 μM CNQX and 30-60 μM APV, respectively. Bicuculline 30 μM was also present to block $GABA_A$ receptors. We recorded from 20 neurons with a mean resting membrane potential (RMP) of -80 mV, mean spike size of 110 mV, and mean input resistance of 40 MΩ. Superfusion of 300 μM acamprosate did not alter RMPs or the input resistance of these neurons. However, acamprosate significantly increased NMDA-EPSPs and -EPSCs in 70% of neurons tested ($n = 10$; $F(1,31) = 7.646$; $p = 0.0096$; 3 neurons showed no effect). In contrast, acamprosate (300 μM) slightly reduced or had no effect on the evoked non-NMDA EPSPs in 7 cells. Washout of acamprosate returned both NMDA and non-NMDA EPSPs to control values. These results are compatible with previous findings in hippocampus (Madamba et al., in submission) and in cortex (Zeise et al., Eur. J. Pharmacol. 231: 47, 1993) suggesting that the clinical efficacy of acamprosate may result from modulation of glutamatergic neurotransmission. *Supported by grants from NIH (AA06420) and Groupe LIPHA (Lyon, France).*

825.14

THE EFFECTS OF PHENCYCLIDINE ON A MULTIPLE SCHEDULE OF ETHANOL AND SACCHARIN SELF-ADMINISTRATION. K.L. Shelton* and R.L. Balster Dept. of Pharmacology/Toxicology, Medical College of Virginia, Richmond, VA 23298-0310

Antagonism of the NMDA subtype of glutamate receptors has been implicated in the discriminative stimulus and reinforcing properties of ethanol. Recent studies have shown that systemic injections of a nonselective glutamate antagonist and intraaccumbens injections of competitive NMDA antagonists will decrease ethanol self-administration. We have developed a multiple schedule of ethanol and saccharin self-administration in which selective drug effects on ethanol-reinforced responding can be demonstrated. Adult male Long-Evans rats were trained using a post-parandial drinking procedure to lever press during daily 60-min sessions for 0.05 ml of 10% ethanol (w/v) and 0.1% saccharin solutions under an alternating fixed-ratio 4 multiple schedule. The present study was designed to examine the ability of repeated administration of the noncompetitive NMDA antagonist, phencyclidine (PCP), to selectively attenuate ethanol self-administration. Ascending doses of 1, 2 and 4 mg/kg of PCP were given i.p. 15 min prior to the self-administration session for six consecutive days. Six-day blocks with pre-session saline injections intervened between testing each PCP dose. Doses of 1 and 2 mg/kg of PCP failed to significantly alter the number of either PCP or saccharin deliveries. The 4 mg/kg dose of PCP significantly decreased both ethanol and saccharin self-administration. Levels of saccharin responding quickly returned to control levels during the saline control sessions following the 4 mg/kg dose of PCP, however ethanol responding remained depressed for at least 10 sessions. These results indicate that systemic administration of PCP does not selectively attenuate ethanol self-administration, but a high dose may have selective effects on relapse. We are currently testing the competitive NMDA antagonist CPPene in the same multiple schedule to determine if it has effects similar to that of PCP. (Research supported by NIAAA grants AA-08473, AA-05357 and NIDA grant DA-01442)

825.15

DISCRIMINATIVE STIMULUS EFFECTS OF THE NOVEL NEUROSTEROID, CO 8-7071, IN PENTOBARBITAL-TRAINED RHESUS MONKEYS. J. K. Rowlett* and W. L. Woolverton. Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS 39216.

Co 8-7071 (3α,21-dihydroxy-3β-triflouromethyl-5β-pregnan-20-one, 21-hemisuccinate) is a water-soluble, orally-bioavailable neurosteroid pro-drug. The purpose of this study was to assess the discriminative stimulus (DS) effects of Co 8-7071 in rhesus monkeys (N=2) trained to discriminate pentobarbital (PB, 10 mg/kg, i.g., 60 min pre-session) from saline. Lever pressing was maintained under a discrete-trials shock avoidance schedule of reinforcement (30 trials/day, 30-sec ITI, FR 5). During test sessions, in which responding on either lever was reinforced, the monkeys were given PB (3.0-17 mg/kg, i.g., 60 min pre-session) or Co 8-7071 (3.0-30 mg/kg, i.g., either 60 or 120 min pre-session). Administration of PB resulted in a dose-related increase (0-100%) in the percentage of responses emitted on the drug-appropriate lever with a dose-related decrease in response rate (resp/sec). Co 8-7071 produced drug-appropriate responding in both monkeys, depending on pre-session time. At 60 min pre-session, Co 8-7071 (3.0-17 mg/kg) produced a dose-related increase (0-100%) in drug-appropriate responding in one monkey, but not in the second monkey. At 120 min pre-session, Co 8-7071 (3.0-30 mg/kg) produced a dose-related increase (0-100%) in drug-appropriate responding in the one monkey tested to date. No systematic effects on response rate were seen after Co 8-7071 treatment. These results suggest that Co 8-7071 may share some DS effects with PB in rhesus monkeys. (Supported by NIDA grant DA-09139).

825.16

BEHAVIORAL PHARMACOLOGY OF NEGATIVELY-PUNISHED RESPONDING IN THE RAT. J.M. Rhoads, T.A. Tatham* . Dept. of Psychiatry, Uniformed Services University of the Health Sciences, Bethesda, MD, 20814.

Behavior may be suppressed by both postive and negative punishment. Positive punishment consists of *presentation* of response-contingent *aversive* events such as shock. In contrast, negative punishment suppresses behavior by response-contingent *removal* of access to *positive reinforcement* (timeout). The effects of CNS drugs on positively-punished behavior have been extensively characterized, while the behavioral pharmacology of negative punishment is virtually unknown. This study examines the effects of drugs on negatively-punished responding with emphasis on indentifying similarities and differences in the effects of drugs on responding punished by negative versus positive punishment.

Initially the lever-pressing of 4 rats was maintained by a fixed-interval (FI) schedule in which the 1st response after 1 min produced a food pellet. Negative punishment was subsequently introduced so that every 5th response produced a timeout during which the response lever was retracted and inoperable, and the 1-min fixed-interval timer was paused. After 1 min the lever was re-extended into the chamber, and timing resumed. Introduction of punishment decreased the reponse rate from .623 responses/sec to .168 responses/sec. Once responding stabilized, drugs were administered before some sessions. The following drugs increased punished responding: the psychomotor stimulant *d*-amphetamine > the sigma and PCP recptor agonist MK-801 (dizolcipine) > the benzodiazepine chlordiazepoxide. The opiate morphine, the barbituate pentobarbital, and the 5-HT1A receptor agonist 8-OH-DPAT failed to reliably increase rates. Results resemble those typically obtained with positive punishment in that responding in both procedures is increased by chlordiazepoxide and MK-801, and 8-OH-DPAT and morphine fail to increase rates. However, effects differ in that pentobarbital increases only positively-punished responding, and *d*-amphetamine increases only negatively punished responding. The results suggest distinct pharmacological profiles for the two forms of punishment despite similarity in their behavioral effects.

825.17

EFFECTS OF CHLORDIAZEPOXIDE AND BUSPIRONE ON BEHAVIOR SUPPRESSED BY PRESENTATION OF TIMEOUT FROM FOOD. F.van Haaren* and K.G. Anderson, Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611-2250.

Six rats were exposed to a two-component multiple schedule. In one component (A), lever presses were followed by pellet presentation on a RI 30-s schedule, in the other component (B), pellet presentation occured on the same schedule, but lever presses also resulted in timeout presentation (10 sec) on a RI 2-s schedule (conjoint RI 30-s food, RI 2-s timeout). The two components were presented in an A-B-A-B-A sequence. Once responding had stabilized subjects were injected with different doses of chlordiazepoxide (CDP, 1, 3, 10, 17 and 30 mg/kg, IP, -15 min), or different doses of buspirone (BUSP, 0.1, 0.3, 1.0, 1.7, 3.0 or 4.2 mg/kg, IP, -15 min). Response rates during the conjoint component of the schedule were considerably lower than those in the other component. Low doses of CDP and BUSP produced a small increase in RI responding. Suppressed responding was greatly increased by low doses of CDP, but hardly affected, or decreased by low doses of BUSP. Higher doses of CDP (= > 10 mg/kg) and BUSP (= > 1.0 mg/kg) suppressed responding in both components of the multiple schedule.

DRUGS OF ABUSE: AMPHETAMINES AND OTHER STIMULANTS V

826.1

EFFECTS OF SYSTEMIC AND INTRACRANIAL CAFFEINE ON DOPAMINE OVERFLOW IN ANESTHETIZED AND FREELY-MOVING RATS: *IN VIVO* MICRODIALYSIS STUDIES.
Enrico Museo* and Agu Pert. Biological Psychiatry Branch, National Institute of Mental Health, Bethesda, MD 20892.

The neurochemical basis of caffeine's psychomotor stimulant actions is not completely understood. The possibility has been raised that as with other psychomotor stimulants (eg. nicotine, amphetamine, and cocaine), the effects of caffeine are in part due to the activation of dopaminergic substrates. We studied the effects of caffeine on extracellular levels of dopamine (DA) in the nucleus accumbens of either anesthetized or freely-moving rats. Dialysate samples were collected at 20-min intervals and DA levels were quantified using HPLC with electrochemical detection. Caffeine was administered systemically (0, 20 or 40 mg/kg, i.p.) or directly into the nAcb (0, 10, 100, 1000 μM). In anesthetized rats, systemic administrations of 40 mg/kg, but not 20 mg/kg, of caffeine increased DA overflow in the n. accumbens. In unanesthetized rats, however, both doses increased locomotor output while having no effect on n. accumbens DA. When administered directly into the n. accumbens caffeine (1000μM) facilitated DA overflow in unanesthetized as well as anesthetized rats. In the former case, caffeine also had a locomotor stimulant effect. Even though caffeine can facilitate DA overflow in the n. accumbens under certain conditions (at high concentrations or under anesthesia) the relevance of such effects to its behavioral actions is not apparent. Because systemic injections of caffeine (20 & 40 mg/kg)had behavioral effects without altering DA overflow, it seems that caffeines behavioral effects are not dependent on the facilitation of DA overflow in the n. accumbens.

826.2

NMDA-TYPE GLUTAMATE RECEPTOR INVOLVEMENT IN CAFFEINE'S LOCOMOTOR STIMULANT EFFECTS AND TOLERANCE TO THESE EFFECTS. K.R.Powell* and S.G.Holtzman Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

The involvement of NMDA-type glutamate receptors in caffeine's locomotor stimulant effects and the development of tolerance to these effects was examined in rats. Rats were administered caffeine and dizocilpine (MK-801), a competitive NMDA receptor antagonist, alone and in combination. Both caffeine and dizocilpine increased dose-dependently locomotor activity in rats. When combined with caffeine, the highest dose of dizocilpine, that alone did not increase locomotor activity (0.1 mg/kg), enhanced the locomotor stimulant effects of caffeine. Lower doses of dizocilpine (0.01-0.03 mg/kg) did not alter the effects of caffeine. During the tolerance phase of the study, rats were exposed to caffeine using scheduled access to a caffeinated drinking solution (1.0 mg/ml) and were implanted with osmotic minipumps (Alzet Corp.) containing either dizocilpine (0.1 mg/kg/day infusion) or saline for a period of 7 days. The effects of caffeine on locomotor activity were examined before, during and following chronic exposure to caffeine to measure the development of tolerance to these effects. Dizocilpine did not alter the development of tolerance to caffeine's effects. These data indicate a supraadditive interaction between the acute effects of caffeine and dizocilpine and suggest that NMDA-type glutamate receptors may be involved in the mechanisms underlying locomotor stimulant effects of caffeine. On the other hand, these data suggest that NMDA-type glutamate receptors are not involved in the development of tolerance to caffeine's locomotor stimulant effects in rats. (Supported by DA 03413)

826.3

VENTRAL PALLIDAL 6-OHDA LESIONS IMPAIR AMPHETAMINE SELF ADMINISTRATION AND LOCOMOTOR ACTIVITY. N.J. DeSousa, J.N. Nobrega[1], and P.J. Fletcher[1]*. Dept. of Psych., Univ. of Toronto, Toronto, Canada, *M5S 1A1*; and [1]Clarke Inst. Psychiatry, Toronto, Canada, *M5T 1R8*.

Recent studies have suggested a role for the ventral pallidum (VP) in drug self administration and locomotor activity. However, the possible contribution to these behaviours of ascending mesopallidal dopamine (DA) neurons has not yet been evaluated. The present experiments sought to examine the role of VP DA in amphetamine (AMP) self administration (SA) and locomotion in rats.

Rats were trained to intravenously self administer AMP (50 µg/kg/infusion) on an FR1 schedule (1hr/day) until response rates had stabilized. Rats were then treated bilaterally with either intra-VP 6-OHDA (2.4 µg/0.3 µl) or ascorbic acid vehicle. Immunohistochemical results from pilot studies have indicated reliable VP DA depletions without affecting DA content in the ventral tegmental area, frontal cortex, or nucleus accumbens. In expt. 1, following a 1 week recovery period, animals were again given access to AMP (50 µg/kg/infusion) and allowed daily 1 hr SA sessions for 10 days. In expt. 2, an AMP (100, 50, 25, 12.5, 6.125, and 0 µg/kg/infusion) dose response analysis was conducted over the subsequent 6 days. Finally, rats were tested for the expression of AMP-induced (1.0 mg/kg ip) locomotor activity in photobeam-based activity monitors in expt. 3.

Results from expt.1 showed that rats treated with 6-OHDA administered significantly fewer amphetamine infusions than control animals over the last 5 days of testing. Further, results from expt. 2 revealed that 6-OHDA treated rats displayed a dampened inverted-U dose response curve, administering significantly fewer infusions across middle range doses. In expt. 3, results indicated that animals treated with 6-OHDA were significantly less responsive to the locomotor activating effects of AMP. These results suggest the involvement of VP DA in the rewarding and locomotor activating effects of psychostimulant drugs.

826.4

CONDITIONED INCREASES IN MOTOR ACTIVITY AND DOPAMINE CONCENTRATIONS IN THE NUCLEUS ACCUMBENS OF THE RAT FOLLOWING REPEATED ADMINISTRATION OF COCAINE OR *d*-AMPHETAMINE. P. Di Ciano, C.D. Blaha and A.G. Phillips* Department of Psychology, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4

One of the terminal regions of the mesotelencephalic dopamine (DA) pathway, the nucleus accumbens (N.Acc.), has been implicated in the primary and secondary reinforcing properties of drugs of abuse. This study demonstrates that repeated pairings of either cocaine or *d*-amphetamine (amph) with a compound environmental stimulus (odour, light) produced conditioned increases in both locomotor activity and extracellular DA concentrations in the N.Acc., when rats were exposed to the compound CS in the absence of the drug. *In vivo* chronoamperometry was used to monitor extracellular levels of DA in the N.Acc. Motor activity was recorded as the number of photocell beam crossings in the test chambers. Compared to controls, both cocaine and amph significantly increased motor activity (mean maximum: 880 and 470 photocell counts, respectively) in the first 10 min after drug administration. Significant increases were also seen in associated DA oxidation currents that plateaued at 2nA and 3.75nA after cocaine and amph administration, respectively. When compared to controls, rats that had previously received either cocaine or amph paired with the CS showed significant increases in motor activity, upon presentation of the compound CS, that were of comparable magnitude (~200/ 10min), for animals treated with either drug. Associated changes in extracellular DA concentrations in the N.Acc. were significantly elevated ~20 min after presentation of the CS, to levels between 2-3nA and 3-4nA for the cocaine and amph-treated rats, respectively. The present experiments provide support for the hypothesis that DA in the N.Acc. is involved in the conditioned incentive effects of drugs of abuse.

826.5

DOSE-DEPENDENT EFFECT OF METHAMPHETAMINE ON NEUROTENSIN RELEASE IN STRIATUM AND NUCLEUS ACCUMBENS. J.D. Wagstaff*, J.W. Gibb and G.R. Hanson. Dept.of Pharmacology & Toxicology, University of Utah, Salt Lake City, UT 84112

Central neurotensin (NT) systems are thought to play an important role in the modulation of dopaminergic pathways. Stimulants of abuse such as methamphetamine (METH) dramatically alter tissue levels and synthesis of this neuropeptide. However, the regulation of NT release has been difficult to study *in vivo* as extracellular levels of NT are extremely low. We previously measured extracellular NT by microdialysis in awake animals, and demonstrated regulation by D-2 receptors. The present study examines the effect of METH on NT release from striatum and nucleus accumbens, and characterizes the role of dopamine D-1 and D-2 receptors in mediating METH-induced alterations. METH altered extracellular NT in a dose-dependent manner. At a low dose (0.5 mg/kg), METH increased NT release from both regions to approximately 200% of control. An intermediate dose (5.0 mg/kg) increased extracellular NT to 150% in the striatum and 140% in the accumbens. At 15 mg/kg, METH had no affect on NT release. The role of DA receptor subtypes was assessed in the striatum by combining selective D-1 (SCH-23390) and D-2 (eticlopride) antagonists with the low and high doses of METH. Eticlopride (0.5 mg/kg) alone decreased NT release ≈20-30% and completely blocked the effect of low dose METH. SCH-23390 (0.5 mg/kg) alone had no effect on basal NT release, but the combination of D-1 blockade with 15 mg/kg METH increased NT release to ≈150% of control. These data indicate that the effect of low dose METH on NT release is mediated through stimulation of D-2 receptors, while the lack of effect of high dose METH is due to D-1 activation. (Supported by USPHS grants DA 34471 and DA 00869)

826.6

INCREASED DOPAMINE D1 RECEPTOR SITES OF THE SUBSTANTIA NIGRA RETICULATA SUBSEQUENT TO INTERMITTENT PRETREATMENT WITH METHAMPHETAMINE. Y.Zhang and J.A.Angulo*, Department of Biological Sciences, Hunter College, NY.

We have assessed the effect of intermittent methamphetamine (METH) pretreatment on haloperidol-induced effects on neostriatal neuropeptide mRNA abundance and dopamine receptor levels. Rats received a total of five METH injections (1.5 mg/kg), one injection every third day. Fifteen days after the last METH injection, rats received daily injections of haloperidol for seven consecutive days. Treatment with METH did not afftect neostriatal ^{3}H-spiperone (dopamine D2) binding sites, but METH pretreatment significantly potentiated haloperidol-induced increases of D2 receptor sites in ventrolateral caudate-putamen (vlCPu) and nucleus accumbens (NAc). In contrast, METH treatment decreased ^{3}H-SCH 23390 (D1 receptor) binding sites in the NAc. Haloperidol treatment of METH-pretreated rats significantly decreased D1 sites in vlCPu, anterior CPu (aCPu) and NAc. In the midbrain ventral substantia nigra reticulata (vSNr), METH pretreatment increased D1 receptor sites (202% above control). In addition, haloperidol treatment of saline-pretreated rats increased D1 receptor sites of the vSNr 58% relative to controls. METH pretreatment enhanced haloperidol-induced changes in neostriatal preproenkephalin (PPE) or preprotachykinin mRNA abundance. The present results show that pretreatment with METH increases dopamine D1 receptor sites in the midbrain substantia nigra pars reticulata and potentiates haloperidol-induced alterations of dopamine receptors and neuropeptide mRNA abundance in the caudate-putamen and nucleus accumbens of the rat brain.

826.7

METHAMPHETAMINE (M)-STIMULATED STRIATAL DOPAMINE (DA) RELEASE AS MEASURED BY MICRODIALYSIS DECLINES RAPIDLY OVER TIME AFTER PROBE INSERTION. B. Gough*, J. Bowyer and R. Holson. National Center for Toxicological Research, Jefferson, AR 72079.

Three experiments were conducted to investigate the striatal response to repeated M exposure in male rats. In all three experiments, M exposure occurred for one group three times at two-hr intervals, with the first exposure occurring 2 hr after probe insertion. Independent of route of administration (1 mg/kg M ip, or 20 µM of M for 10 min via the microdialysis probe), the DA peak elicited by the third M exposure was only 50 % as large as that elicited by the first exposure, 4 hrs earlier. This reduction in the response to M was a function of time post probe-insertion, and not of prior M exposure. When probes were inserted at 0700 hrs but the first M infusion did not occur until 1300 hrs, striatal DA levels were as low as the 1300 hr peak in rats given infusions at 0900, 1100 and 1300 hrs. Conversely, when the probe was inserted 1 hr before the third ip M injection, the DA released by the third injection was as great as that in response to the first injection, measured 2 hr after probe insertion. Finally, ip M-induced DA peaks 24 hr after probe insertion were only 10 % of those seen 2 hr after probe insertion, and these much smaller peaks did not decline over repeated injection. It is concluded that the magnitude of M-stimulated striatal DA release declines rapidly over the first 24 hours following probe insertion.

826.8

METHAMPHETAMINE-INDUCED REGULATION OF STRIATAL AND CORTICAL NMDA RECEPTORS. A.J. Eisch*, S.J. O'Dell and J.F. Marshall, Psychobiology Dept., University of California, Irvine, CA 92717-4550.

Methamphetamine (m-AMPH) produces long-lasting damage to dopaminergic and serotonergic terminals in the striatum and cortex of the rat. Evidence also suggests that m-AMPH is toxic to non-monoaminergic cortical neurons. We used quantitative autoradiography to investigate changes in the dopaminergic and glutamatergic systems in striatal and cortical regions after a neurotoxic regimen of m-AMPH. Rats were given i.p. injections of saline (1 mg/ml) or m-AMPH (4 mg/kg of free base) every two hours for four injections. One week or one month later, regions of the striatum, prefrontal and parietal cortices were examined for m-AMPH-induced changes in [3H]mazindol (MAZ) binding to striatal dopamine (DA) transporters and [3H]glutamate (GLU) binding to NMDA receptors. One week after m-AMPH, both [3H]MAZ binding and [3H]GLU binding to NMDA receptors were decreased in striatal subregions. Specifically, the ventral and lateral sectors of the striatum showed the greatest decreases in [3H]MAZ and [3H]GLU binding, while the nucleus accumbens showed no significant m-AMPH-induced damage. One month after m-AMPH, striatal [3H]MAZ binding was still significantly decreased, while NMDA receptors had returned to control levels. The parietal cortex also showed m-AMPH-induced changes. One week after m-AMPH, a significant *increase* in [3H]GLU binding to NMDA receptors was seen in layers II/ III and IV. One month after m-AMPH, only layers II/III showed a significant increase in [3H]GLU binding. In contrast, regions of the prefrontal cortex showed no changes in NMDA receptor density either one week or one month after m-AMPH. This is the first demonstration that forebrain NMDA receptors are altered by a neurotoxic regimen of m-AMPH. These results support the view that repeated m-AMPH adminstration affects excitatory amino acid transmission in selected cortical and striatal regions.

826.9

METHAMPHETAMINE EFFECTS ON BRAIN ENERGY METABOLISM: COMPARISON BETWEEN EXTRACELLULAR LACTATE IN STRIATUM AND PREFRONTAL CORTEX. S.E. Stephans*, T.S. Whittingham, A.J. Douglas and B.K. Yamamoto Departments of Psychiatry, Neuroscience, and Neurological Surgery, Case Western Reserve Univ., Cleveland, OH 44106.

A high dose of methamphetamine (METH) can lead to depletion of tissue dopamine in the striatum (STR). In contrast, the medial prefrontal cortex (PFC) is relatively resistant to the toxic effects of METH. The mechanisms underlying this selective effect are unknown; however, energy depletion has been linked to METH-induced decreases in striatal dopamine content. To further extend this finding, microdialysis coupled with an enzymatic fluorometric assay was utilized to assess *in vivo* the dynamic changes in extracellular lactate concentrations after METH in awake rats. Lactate concentration in the dialysate was measured and used as an index of possible energetic stress in STR and PFC during a neurotoxic dosing regimen of METH (10.0 mg/kg, i.p., every 2 hours for a total of 3 injections) or saline. There was a significant increase (228% of baseline) in extracellular lactate in STR over time beginning immediately after the first METH injection and persisting up to 4 hours after the third injection ($p<0.02$). There was no significant change in striatal lactate over time in saline injected rats. Extracellular concentrations of lactate in the PFC were not significantly different between METH and saline injected rats. DA tissue content measured 4 days following administration of METH was significantly depleted in STR but not PFC ($p<0.05$). Overall, these data are evidence that METH enhances energy consumption more in the STR than in the PFC. This effect may be due to the greater density of DA terminals in STR. It is possible that the acute and sustained enhancement in energy utilization is related to the long-term dopamine depletions produced by METH.

826.10

BEHAVIORAL SENSITIZATION TO 7-OH-DPAT: EFFECTS OF SELECTIVE DOPAMINE ANTAGONISTS. B.A. Mattingly*, S. Fields, M. Langfels, M. Cecil, & D. Giovannini. Department of Psychology, Morehead State Univ., Morehead, KY 40351.

Repeated treatments with the dopamine (DA) D_2-type agonists, bromocryptine and quinpirole, result in the development of behavioral sensitization. This sensitization effect, however, may be prevented by the co-administration of either DA D_1 or D_2 antagonists. The primary purpose of this study was to determine whether DA D_1- and D_2-type antagonists would also block the development of sensitization to the putative DA D_3 agonist, 7-OH-DPAT. In Exp. 1, rats were tested for 2 hrs in photocell activity arenas daily after treatment with 7-OH-DPAT (0, .01, .10, or 1.0 mg/kg). All doses initially inhibited activity, but with repeated treatment, the 1.0 mg/kg dose resulted in sensitization. No cross-sensitization to cocaine was observed. In Exps. 2 & 3, rats were co-administered 7-OH-DPAT (1.0 mg/kg) and either the D_2-type antagonist, eticlopride (ETIC, 0.3 mg/kg) or the D_1-type antagonist, SCH 23390 (SCH, 0.2 mg/kg), and tested for activity. Both antagonists significantly suppressed activity and prevented the progressive 7-OH-DPAT-induced increase in activity over sessions. ETIC, but not SCH, also blocked the development of sensitization. That is, after a 7-OH-DPAT challenge injection, rats pretreated with ETIC and 7-OH-DPAT did not differ from vehicle pretreated rats. Rats pretreated with SCH and 7-OH-DPAT, however, were significantly more active than rats pretreated with only 7-OH-DPAT.

826.11

THE D_3 AGONIST 7-OH-DPAT GENERALIZES TO THE DISCRIMINATIVE STIMULUS EFFECTS OF AMPHETAMINE IN RATS. R. A. Bevins*, M. C. Bradley, J. E. Klebaur, & M. T. Bardo. Psychology Dept, Univ of Kentucky, Lexington, KY 40506-0044.

Rats were first trained to discriminate between 1 mg/kg amphetamine and saline (ip) using a two-lever drug discrimination procedure. Following acquisition of the discrimination, generalization to a range of amphetamine doses (0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/kg) was examined in 4-min extinction tests. The degree of amphetamine-appropriate responding varied directly with the dose of amphetamine. Response rates decreased systematically with greater amphetamine doses. We then assessed the ability of the putative D_3 agonist 7-OH-DPAT (0, 0.01, 0.03, 0.1, 0.3, and 1.0 mg/kg) to occasion amphetamine-correct responding. In a balanced manner, all rats were tested twice with each dose; on one test the 7-OH-DPAT was injected ip and in the other test it was injected sc. Regardless of injection route, the higher doses of 7-OH-DPAT fully generalized to amphetamine. However, 7-OH-DPAT injected sc occasioned greater amphetamine-correct responding at lower doses than when injected ip. 7-OH-DPAT also produced a dose-dependent decrease in barpress rates with both injection routes. The D_2 antagonist eticlopride (0.01 and 0.05 mg/kg; ip) partially blocked the amphetamine-appropriate responding induced by 7-OH-DPAT. This latter results argues that the generalization of 7-OH-DPAT may be mediated, at least in part, by dopamine D_2 receptors.

826.12

DOSE-DEPENDENT EFFECTS OF THE D3-PREFERRING AGONIST 7-OH-DPAT. J. L. Neisewander*, T. V. Khroyan, and D. A. Baker. Department of Psychology, Arizona State University, Box 871104,Tempe, AZ 85287-1104.

Dose-dependent effects of 7-OH-DPAT (DPAT) on motor behaviors and conditioned place preference (CPP) were assessed. Two-day conditioning trials were conducted. On one day, animals received one of 8 doses of DPAT (0-5 mg/kg, s.c) and were placed into a distinct compartment for 40 min. On the other day, they were injected with saline and immediately placed into a different compartment for 40 min. Three trials were conducted over consecutive days. Locomotion, sniffing, and yawning were measured following the first and last injection of DPAT. CPP was then assessed by recording the amount of time animals spent in each compartment when given free-access to both for 15 min. DPAT produced a U-shaped dose-dependent change in locomotion and sniffing with the greatest decreases observed at 0.01 and 0.03 mg/kg. DPAT also produced an inverted U-shaped dose-dependent change in yawning with the greatest increases observed at 0.03 and 0.1 mg/kg. DPAT-induced yawning was sensitized at a dose of 0.1 mg/kg. The 5 mg/kg dose of DPAT produced CPP. None of the other doses (0.003-1 mg/kg) produced CPP, and in fact there was a trend for place aversion at 0.3 mg/kg. DPAT has a 100-fold greater affinity for D3 receptors relative to D2 receptors. Thus, it is possible that the low doses (0.01-0.1 mg/kg) that increased yawning and decreased locomotion and sniffing may preferentially occupy D3 receptors. Furthermore, these putative D3-preferring doses of DPAT do not produce CPP and may produce place aversion. (Supported by DA07730)

826.13

EFFECTS OF 7-OH-DPAT ON AMPHETAMINE-INDUCED STIMULANT BEHAVIORS AND CONDITIONED PLACE PREFERENCE. T. V. Khroyan *, D. A. Baker, R. A. Fuchs and J. L. Neisewander. Department of Psychology, Arizona State University, Box 871104, Tempe, AZ 85287-1104.

Putative D3-preferring doses of 7-OH-DPAT (0.01-0.1 mg/kg) produce a decrease in locomotion and sniffing and do not produce conditioned place preference (CPP). This study examined the effect of these doses on amphetamine-induced behaviors and CPP. Three 2-day conditioning trials were conducted over consecutive days. On one day of each trial, animals received an injection of either amphetamine (1.0 mg/kg) coadministered with 7-OH-DPAT (0- 0.1 mg/kg) or saline and were placed into a compartment for 40 min. On the other day, animals were injected with saline and placed into a different compartment for 40 min. Locomotion, sniffing, and headbobbing were measured following the first and third drug injections. Following conditioning, CPP was assessed by recording the amount of time animals spent in each compartment when given access to both. Amphetamine increased locomotion after the first injection, and this effect was attenuated by 0.03 mg/kg 7-OH-DPAT. Amphetamine-induced locomotion was decreased dose-dependently by 7-OH-DPAT after the third injection due to the emergence of dose-dependent headbobbing, a behavior not observed in animals receiving amphetamine alone. Amphetamine coadministered with 0-0.03 mg/kg 7-OH-DPAT produced CPP, whereas amphetamine coadministered with 0.1 mg/kg 7-OH-DPAT did not produce CPP. These results suggest that 7-OH-DPAT potentiates the stimulant properties of amphetamine and alters the rewarding properties (supported by DA07730 and HHMI).

826.14

REPEATED PHENCYCLIDINE TREATMENTS ACTIVATE CHOLECYSTOKININ SYSCTEMS IN RAT BRAINS. H.Shibuya*1, K.Yamada1, T.Yoshikawa1, T.Nabeshma2, M.Toru1, 1;Department of Neuropsychiatry, Tokyo Medical and Dental Univ., 1-5-45, Yushima, Bunkyo-ku, Tokyo, 113, Japan, 2;Nagoya Univ., School of Medicine, 45, Tsurumacho, Showa-ku, Nagoya, 466, Japan,

Reported findings on cholecystokinin(CCK) immunoreactivity in the postmortem brain and cerebrospinal fluind, CCK receptor bindings and clinical therapeutic trials with CCK related substances suggest functional alterations of CCK systems in the schizophrenic brain. Meanwhile, phencyclidine(PCP) is known to produce positive and negative psychotic symptoms in human. We assumed the PCP treated rat as the schizophrenic animal model and determined the CCKmRNA and CCK immunoreactivities in various brain areas in order to investigate the functional state of CCK in schizophrenia. Single administration of PCP (7.5mg/kg,ip) promptly and transiently decreased CCK immunoreactivities in many brain areas and CCKmRNA in the hippocampus and parieto-occipital cortex. However, repeated administrations of PCP increased the synthesis of CCK. These findings would suggest that CCK systems are hyperactive in schizophrenic brain and play roles to produce some schizophrenic symptoms.

826.15

ACUTE AND LONG-TERM NEUROCHEMICAL EFFECTS OF METHCATHINONE, A NEW STIMULANT OF ABUSE. M.P. Gygi, J.W. Gibb, and G.R. Hanson.*Dept. of Pharmacology and Toxicology, Univ, of Utah, Salt Lake City, UT 84112.

Methcathinone (CAT), a synthetic derivative of cathinone, emerged as an illicit drug of abuse in the U.S. during the early 1990s. Because of ease of synthesis, CAT is preferred by some over cocaine or methamphetamine (METH). Multiple doses of CAT were shown to be toxic to brain dopamine (DA) and 5-hydroxytryptamine (5HT) neurons (Martello et al., Soc Neurosci 419.15, 1994). In striatum, a single dose of CAT decreases the activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme in the synthesis of 5HT. To characterize further the time course of these effects, rats were given 4 doses of CAT (30 mg/kg) 4 h apart, and sacrificed 72 h or 30 d following the last dose. In the 72 h group, striatal TPH activity was decreased. The activity of striatal tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, was also decreased. Striatal concentrations of DA and 5HT and their metabolites were reduced as well. Both TH and TPH activites returned to control by 30 d after treatment. Because drug-induced increases in mesostriatal dopaminergic activity elevate striatal levels of neurotensin, the response of this peptide to CAT was assessed and compared to METH, a known releaser of DA. Rats were given either 4 doses of CAT (30 mg/kg), METH (15 mg/kg) or saline 4 h apart, and sacrificed 18 h after the last dose. Both METH and CAT increased striatal levels of neurotensin to greater than 200% of control. These data demonstrate that CAT affects monoaminergic parameters in the striatum. These effects do not appear to be permanent. Since striatal neurotensin levels were elevated by CAT, it is possible that the neurochemical response is due to CAT-induced DA release. (supported by DA 04222 & DA 00869)

826.16

S(-)METHCATHINONE AS A DISCRIMINATIVE STIMULUS: EFFECTS OF OTHER CNS STIMULANTS.
R. Young* and R.A. Glennon. Dept. Med. Chem., MCV/VCU, Richmond, VA 23298

Methcathinone ("Cat") is a CNS stimulant that is a very significant drug of abuse in the former Soviet Union. It also has appeared on the clandestine market in the US and has been recently classified as a Schedule I substance. We have shown[1] previously that racemic methcathinone and its two optical isomers all produce both amphetamine- and cocaine-like discriminative stimulus effects in rats trained to discriminate S(+)amphetamine and cocaine, respectively, from saline. In those studies, S(-)methcathinone was almost twice as potent as S(+)amphetamine and more than eight times more potent than cocaine. In the present study, using rats, S(-)methcathinone (0.5 mg/kg, i.p., 15 min. pretreatment) was employed as the training drug in a two-lever discrimination task. Once established, the S(-)methcathinone stimulus was shown to have a rapid onset to action (5 min pre-session injection interval=89% drug-lever responding) and a duration of effect of approximately 90-120 min. In tests of stimulus generalization, the S(-)methcathinone-stimulus (ED50=0.11mg/kg) generalized to R(+)-methcathinone (ED50=0.39 mg/kg), S(+)amphetamine (ED50=0.20 mg/kg), and cocaine (ED50=1.47 mg/kg). Thus, the present results re-confirm our previous conclusion that S(-)methcathinone is a very potent CNS stimulant with amphetamine- and cocaine-like effects. (DA-01642)[1]Pharm. Biochem. Beh., 50, 601-606, 1995.

826.17

PHARMACODYNAMIC ANALYSIS OF KETAMINE ACTION IN SCHIZOPHRENIA. D. Medoff*, A.C. Lahti, H.H. Holcomb, M. Zhao, C.E. Priebe, C.A. Tamminga. MPRC, University of Maryland School of Medicine, and the Department of Mathematical Sciences, Johns Hopkins University.

The noncompetitive NMDA antagonist ketamine produces a short lived discrete activation of psychotic symptoms in schizophrenia. To study regional neural activity that corresponds to ketamine administration, we administered .3mg/kg of ketamine to five medicated schizophrenic inpatients and measured regional cerebral blood flow (rCBF) using $H_2{}^{15}O$ and positron emission tomography (PET) before (x3) and at seven time points after ketamine administration. The post ketamine administration time points ranged from 6 to 66 minutes. After image registration, a 12 mm three dimensional Gaussian filter was used to smooth the data. Data were analyzed using the Statistical Parametric Mapping (SPM94) software program, MRC Cyclotron Unit, Hammersmith Hospital. Significant patterns of blood flow change over time were detected in several regions. The lingual gyrus shows an immediate drop in blood flow at six minutes and a swift return to baseline by sixteen minutes. The hippocampus also shows an immediate drop in blood flow, but a more gradual return to baseline at 56 minutes. The anterior cingulate, inferior frontal cortex, thalamus, and the cerebellum all showed increases in blood flow, but in distinctly different patterns. It is noteworthy that the areas of ketamine-altered rCBF are circumscribed, limited in number, and much more restricted than the distribution of NMDA/PCP receptors. Based on the localization of blood flow response to ketamine in these subjects, we suggest that limbic brain regions are important in mediating the behavioral actions of ketamine. These data implicate an abnormality of glutamatergic-transmission in psychosis.

826.18

AMYGDALA LESIONS THAT BLOCK SENSITIZATION TO BROMOCRIPTINE FAIL TO BLOCK SENSITIZATION TO BROMOCRIPTINE PLUS MK-801. W. A. Carlezon, Jr.*, F. A. Guarraci, and R. A. Wise Center For Studies in Behavioral Neurobiology, Concordia Univ., Montréal, QC, CANADA H3G 1M8

Repeated intermittent administration of the dopamine (D_2) agonist bromocriptine (BRO; 5.0 mg/kg, IP) causes progressive increases in sensitivity to the locomotor-stimulating actions of the drug in rats; this "sensitization" is context-specific, such that rats repeatedly given BRO in one environment show no evidence of sensitization when tested in a different environment. We now report that electrolytic lesions of the basolateral amygdala eliminate BRO sensitization without altering sensitivity to the acute stimulant effects of the drug, suggesting that the drug-environment associations that contribute to BRO sensitization are processed at some level in the amygdala. Similar lesions of the amygdala fail to block the progessive increases in locomotion that occur when MK-801 (0.25 mg/kg, IP) is co-administered with BRO, under conditions that do not produce sensitization to MK-801 alone. However, there is no evidence of sensitization when animals (lesioned or sham-lesioned) accustomed to receiving the combination of MK-801 plus BRO subsequently receive either drug alone. Thus it is the *absence* of MK-801 in animals accustomed to the combination of drugs that precludes expression of sensitization. These findings suggest that MK-801 makes responses learned under its influence "discriminative cue-specific", and that such cues can facilitate experience-dependent changes in drug sensitivity even when the amygdala is damaged.

826.19

FASCICULUS RETROFLEXUS LESIONS INCREASE SELF-ADMINISTRATION OF AMPHETAMINE. Carol A. Murphy and Marion Murray*. Department of Anatomy and Neurobiology, Medical College of Pennsylvania and Hahnemann University, 3200 Henry Avenue, Philadelphia, 1PA 19129.

Disruption of the efferent pathway of the habenular nuclei, the fasciculus retroflexus (FR), produces behavioral and physiological changes that reflect a state of increased arousal, including increased open field activity and chronically elevated basal levels of corticosterone. Within a normal population of Sprague-Dawley rats, similar responses to stress are positively correlated with increased self-administration of amphetamine. Because habenular projections through the FR tonically inhibit the activity of dopaminergic (DA) neurons in the ventral tegmentum, and intake of psychostimulant drugs is tightly linked to forebrain DA activity, we tested whether rats with FR lesions would self-administer higher doses of amphetamine. Two months after receiving either FR or sham lesions, female Sprague-Dawley rats were prepared with catheters in the external jugular vein and tested for intravenous self-administration of d-amphetamine sulfate (10ug/injection) in both nose-poke and lever-press operant response paradigms. Rates of amphetamine self-injection and the incidence of behavioral stereotypy were assessed during daily 1-hour sessions. Our results show that FR-lesioned animals initially self-administered greater amounts of amphetamine than sham controls over the first several days of testing but that their drug intake gradually decreased to near-control levels. Stereotypic responses to amphetamine were also greater after FR lesion and remained elevated with respect to controls for the duration of the experiment. These results suggest that habenular regulation of DA reward systems may normally provide an important negative feedback mechanism which moderates the intake of psychostimulant drugs. Supported by NIMH award MH53635-01.

827.1

SINGLE-UNIT RESPONSES OF HIPPOCAMPAL CA1 PYRAMIDAL NEURONS TO 5-HT1A AGONIST DRUG ADMINISTRATION IN UNANESTHETIZED, UNRESTRAINED RATS. N.Ueda[1], K. Tada[1]*, K. Kasamo[1], T. Kojima[1] and K. Ishikawa[2]. Dept. of Neuropsychiat[1]. and pharmacol[2]., Nihon Univ. Sch. of Med., 30-1 Oyaguchi Kamimachi Itabashi, Tokyo 173, Japan.

Previous electrophysiological experiments demonstrated that 5-hydroxytryptamine (5-HT)1A agonists inhibited spontaneous pyramidal cell activity in anesthetized rats. Since all the 5-HT1A agonists thus far tested act as partial agonists post synaptically, it is important to know whether these 5-HT1A agonists inhibit hippocampal pyramidal cell activity under conditions in which 5-HT neurons are activated. So we examined the effects of 5-HT1A agonists on single-unit activity of hippocampal CA1 pyramidal neurons in unanesthetized,unrestrained rats. Subcutaneous administration of the selective 5-HT1A agonists, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), buspirone, ipsapirone produced a dose-dependent inhibition of hippocampal CA1 pyramidal cell activity. The order of potency was 8-OH-DPAT (ED50 for 8-OH-DPAT=0.07 μ g/kg)> buspirone(ED50 for buspirone=0.19 μ g/kg) which is in agreement with previously reported behavioral, electrophysiological and biochemical studies. The 5-HT1A antagonist, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido) butyl]piperazine (NAN190) inhibited the effect of buspirone(1mg/kg) on single-unit activity of hippocampal CA1 pyramidal neurons. Present results provides further evidence for post synaptic site of action of anxiolytic 5-HT1A agonists.

827.2

MKC-242, A NOVEL 5-HT1A RECEPTOR AGONIST, REPRESENTS DIFFERENT PROFILES FROM AZAPIRONES IN THE ANIMAL MODELS.
M. Abe, R. Tabata, #H. Nakai, #T. Komatsu, K.-I. Saito* and M. Egawa. Pharmaceuticals Lab. I, # Pharmacokinetics and metabolism Research Lab., Mitsubishi Chemical Co., Yokohama Research Center, Yokohama, 227.

MKC-242 (5-[3-[[(2S)-1,4-benzodioxan-2-ylmethyl]amino]propoxy]-1,3-benzodioxol HCl) is a potent and selective 5-HT1A receptor agonist. It showed anxiolytic-like and antidepressant-like effect at very low doses (0.0625-3 mg/kg, p.o.) in some animal models. MKC-242 is structuraly differed from azapirones. In the present study, we investigated two different profiles of MKC-242 from azapirones.

First, the effect of MKC-242 on the immobility time in the forced swimming test in male rats was investigated comparing with buspirone and tandospirone. MKC-242 (0.3-3 mg/kg, i.p.) reduced the immobility time. However buspirone and tandospirone did not. The effect of MKC-242 was antagonized by administration of WAY100135, a 5-HT1A receptor antagonist. In addition, the effect was also antagonized by administration of 1-(2-pyrimidinyl)piperazine, a major metabolite of azapirones.

Second, the effect of MKC-242 on the shock induced fighting behavior in male mice was investigated comparing with the reference compounds. MKC-242 reduced the fighting behavior in a dose dependent manner and the effect lasted for 3 hrs at non-sedative doses (ED50 = 1.7-5 mg/kg,p.o.). Buspirone and tandospirone also reduces the fighting behavior 1 hr after the administration at non-sedative doses (ED50 = 42 and 80 mg/kg, p.o., respectively). However, these compounds were effective 2 hrs after the administration at only sedative doses (ED50 = 160 and 320 mg/kg, p.o., respectively). The serum AUC for MKC-242 was about 400-fold higher than that for buspirone in rats.

In conclusion, MKC-242 showed more potent antidepressant-like effect and longer-lasting anxiolytic-like effect comparing with buspirone and tandospirone. These characters might be due to the structural difference and pharmacodynamic difference from azapirones.

EFFECTS OF CHRONIC AZAPIRONE TREATMENT ON SEROTONERGIC SYSTEMS. G. K. Matheson*, C.Michel, M. Weiberg, and J. Thomas. Neurobiology Laboratory, Indiana University School of Medicine. Evansville, IN 47712.

Buspirone or ipsapirone (3 mg/kg, b.i.d., *s.c.*) was administered to Long-Evans rats for ten days. On day ten, the effects of various concentrations of buspirone or ipsapirone on the inhibition of spontaneous single-unit neural activity or the hypothalamic-pituitary adrenal axis (HPAA) were tested. Acute animals did not receive the drug pretreatment. Serotonergic neurons generating single-unit activity were identified by their location, discharge frequency (0.5-2.5 spikes/sec), wave-form characteristics, and spontaneous activity. Drug effects were determined by comparing the average rate of discharge recorded over one minute before drug administration with the rate recorded during the sixth post-injection one minute interval. In the chronic and single treatment studies, buspirone and ipsapirone were equally efficacious and totally inhibited the spontaneous activity of serotonergic single-unit neurons in a dose-dependent manner. For buspirone the potency (ED_{50} value) was 134 µg/kg for the acute study and 1800 µg/kg for the chronic study, a thirteen fold increase in the required dose. For ipsapirone the ED_{50} value was 700 µg/kg for the acute study and 1200 µg/kg for the chronic study. Chronic buspirone or ipsapirone administration increased the tolerance of the HPAA following a challenge by each drug. The ED_{50} for elevation of plasma corticosterone levels was increased from 4.0 to 7.6 mg/kg for buspirone and 6.2 to 8.0 mg/kg for ipsapirone. The data suggests chronic administration may lead to possible differences in ligand-receptor affinity, metabolic rates, and/or the cyclic AMP-G-protein mechanism.

827.4

CP-93,393, A NOVEL ANXIOLYTIC/ANTIDEPRESSANT AGENT WITH BOTH 5-HT1A AGONIST AND ALPHA-2 ADRENERGIC ANTAGONIST PROPERTIES: *IN VITRO* STUDIES. A.W. Schmidt*, C.B. Fox, J. Lazzaro, S. McLean, A. Ganong, D.W. Schulz, K. Desai, G.M. Bright and J. Heym. Central Research Division, Pfizer Inc., Groton, CT. 06340.

CP-93,393, ((7S,9aS)-2-(2-pyrimidinyl)-7-(succinimido-methyl) 2,3,4,6,7,8,9,9a-octahydro-1H-pyrido[1,2-a]pyrazine), is a novel anxiolytic/antidepressant agent that combines 5-HT1A agonist activity and α2 adrenergic antagonist features. Like buspirone, CP-93,393 lacks affinity for benzodiazepine receptors and would therefore not have the sedative and/or addictive properties of anxiolytics such as diazepam and alprazolam. In rats, CP-93,393 potently inhibits dorsal raphe firing by activating presynaptic 5-HT1A autoreceptors (Reynolds,L.S. et al., this meeting). CP-93,393 binds with moderate affinity (Ki = 100 nM) to postsynaptic 5-HT1A receptors, but displays differential efficacy as an agonist depending on the tissue preparation. For example, adenylate cyclase studies reveal CP-93,393 to be a partial agonist (67% compared to 5-HT, EC50 = 600 nM) in HeLa cells expressing human 5-HT1A receptors, while both CP-93,393 and buspirone behave as full agonists when guinea pig hippocampal membranes are utilized. In recordings of 5-HT mediated hyperpolarizations from CA1 neurons in guinea pig hippocampal slices, CP-93,393 displayed partial agonist activity (52% effect compared to 5-HT, EC50 = 1.8 µM). In addition, CP-93,393 binds with appreciable affinity to human α2A, α2B and α2C adrenergic receptors transfected in CHO cells (Ki = 10 - 50 nM). In adenylate cyclase studies in the α2A clone, CP-93,393 (1 µM) produces a competitive rightward shift of the UK-14,304 dose-response curve, indicating that it acts as an α2A adrenergic antagonist. CP-93,393 has a unique combination of 5-HT1A agonist and α2 adrenergic antagonist properties that may lead to improved efficacy as an anxiolytic/antidepressant agent.

827.5

IN VIVO PROFILE OF CP-93,393: EVIDENCE OF COMBINED 5HT1A AGONIST AND α2 ANTAGONIST ACTIVITIES. L.S.Reynolds*, J.P.Braselton, J.S.Sprouse, H.Rollema, T.Clarke, S.McLean, W.Horner, and J.Heym. Central Research Division, Pfizer Inc. Groton CT. 06340

CP-93,393 is a novel anxiolytic/antidepressant compound possessing both $5HT_{1A}$ and α2 adrenergic properties. In recordings from rat dorsal raphe, CP-93,393 was a potent agonist at the $5HT_{1A}$ autoreceptor, inhibiting the spontaneous firing of serotonergic neurons in a dose dependent manner (ED_{50} =14 µg/kg i.v.) while displaying only moderate affinity for the $5HT_{1A}$ receptor (K_i=92±6 nM, rat cortex). In keeping with its action as a potent presynaptic agonist, this compound also produced a dose-dependent decrease in extracellular serotonin (ED_{50}=1.6 mg/kg s.c.) and 5-hydroxy indole acetic acid (5HIAA) levels as measured by *in vivo* microdialysis in rat hippocampus. In addition to affecting the $5HT_{1A}$ receptor, CP-93,393 also acted as an α2 ligand with significant binding affinity for α2 adrenoreceptors in rat (K_i=35±6 nM). As expected with α2 antagonist activity, levels of the NE metabolite 3-methoxy-4-hydroxy-phenylglycol (MHPG) were increased dose-dependently in rat forebrain. In addition, the spontaneous firing rate of NE-containing neurons in rat locus coeruleus was increased by CP-93,393, possibly through an α2 mechanism. This combination of decreased serotonergic activity and increased NE transmission produced by CP-93,393 could be effective in the treatment of anxiety and affective disorders.

827.6

ANXIOLYTIC ACTIVITY OF CP-93,393: ENHANCED EFFICACY VIA COMBINED 5-HT1A AGONIST AND α2-ADRENERGIC ANTAGONIST ACTIVITIES. P.A. Seymour*, K. Desai and G.M. Bright. Departments of Neuroscience and Medicinal Chemistry, Central Research Division, Pfizer Inc, Groton, CT 06340.

The anxiolytic/antidepressant, CP-93,393, is a 5-HT1A agonist that is both chemically and biologically distinct. Unlike members of the buspirone class its novel azabicyclic structure incorporates α2-adrenergic antagonist activity into the parent molecule. CP-93,393 was discovered using a modification of the Vogel conflict test designed to identify compounds with improved efficacy. After i.p. administration CP-93,393 showed a MED comparable to gepirone (1.78 mg/kg), but yielded much greater efficacy (780 vs 320% of control), which was comparable to that observed with diazepam. Since α2-antagonists have also been shown to have anticonflict activity in a similar paradigm (Gower and Tricklebank, 1988), the interaction between 5-HT1A agonism and α2-antagonism was investigated. It was found that subthreshold doses of the α2-antagonist, idazoxan (IDZ), significantly enhanced the anticonflict effects of submaximal doses of CP-93,393, other 5-HT1A agonists, and benzodiazepines. Moreover, it was found that 8-OH-DPAT, which was inactive at any dose tested when administered alone, elicited robust activity when combined with subthreshold doses of IDZ, indicative of synergy between these two compounds. These data support the hypothesis that the enhanced efficacy observed with CP-93,393 may be due to the combination of 5-HT1A agonist and α2-adrenergic antagonist activities, and suggest that CP-93,393 may show improved efficacy in the clinic as well.

827.7

ADAPTATION OF NMDA RECEPTORS IN ANIMAL MODELS OF DEPRESSION: CHRONIC MILD STRESS (CMS). G.N. Nowak*[1], M. Papp[2] and I. A. Paul[1]. Lab. Neurobehav. Pharmacol. & Immunol., Depts. of Psychiatry and Pharmacology, Univ. of Miss. Med. Ctr., Jackson, MS 39216, USA[1] and Inst. of Pharmacology, Pol. Acad. of Sci., Krakow, Poland[2].

NMDA receptor antagonists are as efficacious as clinically active antidepressants (AD) in both preclinical behavioral paradigms sensitive to ADs and in animal models of depression. Moreover, 10-14 d. continuous administration of ADs is required to produce a dose-dependent adaptation of the NMDA receptor complex in rodents. Specifically, chronic AD administration results in a 2-4 fold reduction in the potency of glycine to displace [^{3}H]5,7-dichlorokynurenic acid from the strychnine-insensitive glycine recognition site of the NMDA receptor complex. In addition, chronic AD treatment results in a 40-100% reduction in the proportion of high affinity, glycine-displaceable [^{3}H]CGP-39653 binding to the glutamate recognition site of the NMDA receptor complex. These effects are not observed following chronic administration of structurally related non-AD treatments. A limitation of these earlier studies is that adaptation of the NMDA receptor complex was observed following AD administration in naive animals. We therefore tested the hypothesis that animal models of depression would result in AD-reversible adaptation of the NMDA receptor complex. We administered imipramine (IMI - 10 mg/kg x 28 days) to rats subjected to CMS. As previously reported, 28 days CMS resulted in reduced consumption of a sucrose solution. This was reversed by administration of IMI. Moreover, we now report that CMS results in adaptation of the NMDA receptor complex and that this adaptation is reversible by chronic IMI treatment. These data lend support to the hypothesis that AD activity is associated with adaptation of the NMDA receptor complex and that "depression" or "anhedonia" in animals is associated with an AD-reversible dysfunction of the NMDA receptor complex.

827.8

SAFETY AND EFFICACY OF THE CCK_B ANTAGONIST CI-988 IN GENERALIZED ANXIETY DISORDER John J. Sramek, Jerome R. Costa, Judith Bammert Adams, Alison MacPherson, and Neal R. Cutler*. California Clinical Trials, Beverly Hills, CA 90211 and Parke-Davis Division of Warner-Lambert, Ann Arbor, MI

CI-988 is a novel peptoid CCK antagonist with affinity for brain CCK_B and gastrin receptors. This study investigated the efficacy of this compound in patients with generalized anxiety disorder (GAD). We report results from one site (n=32) in a multicenter study (n=88). Sixteen patients who had had at least a six-month duration of symptoms were randomized to 300 mg/day CI-988 and 16 to placebo. Twenty-nine patients completed all four weeks of study treatment. The primary efficacy measures were the Hamilton Rating Scale for Anxiety (HAM-A) and the Clinical Global Impression (CGI) scale. Patients on CI-988 showed a greater decrease (p=0.06) in HAM-A scores than did patients on placebo at the end of the study, with mean change in HAM-A total at week 4 of -7.69 (-32.0%) for CI-988 and -4.19 (-18.6%) for placebo. The HAM-A change favoring CI-988 was primarily due to improvement in somatic symptoms of anxiety. There were no significant differences on the CGI between the patients treated with CI-988 versus placebo (mean changes of -0.88 and -0.34, respectively, in impression of severity score, and mean changes of 0.92 and 0.94, respectively, in impression of change scores). All adverse events were rated mild or moderate. One patient on CI-988 discontinued due to moderate abdominal pain; two patients on placebo discontinued for personal reasons. Our findings indicate that CI-988 given for four weeks has a potential anxiolytic effect, though the small sample size limits any certainty. These findings were not reflected in the overall multicenter results; one center favored placebo and the other favored neither group. Given the acceptable tolerability of CI-988 seen here, testing of higher oral doses in patients with GAD may be warranted.

827.9

LYSOSOMOTROPIC DRUGS SLOWLY ACCUMULATE IN HUMAN BRAIN TISSUE. RELATIONSHIP TO THERAPEUTIC LATENCY OF ANTIDEPRESSANT AND NEUROLEPTIC DRUGS? J. Kornhuber*, W. Retz, P. Riederer. Dept. of Psychiatry, University of Würzburg, 97080 Würzburg, Germany.

The mechanism of therapeutic latency of antidepressant (AD) and neuroleptic (NL) drugs is not clearly understood. Current hypotheses include slow adaptive processes after fast access to primary drug targets. Here, we present a hypothesis explaining therapeutic latency by slow accumulation of the drugs in acidic intracellular compartments. We have studied the pharmacokinetics of amantadine, a lysosomotropic model substance. It's fast therapeutic response is mediated by fast access to cell surface receptors. However, it slowly accumulates intracellularly in human brain tissue. Half-maximal and plateau concentrations are reached after 8 and at least 70 days of treatment, respectively. The concentration in brain tissue relative to CSF and serum is about 20:1. The high storage capacity of brain tissue is probably related to lysosomotropic properties of amantadine. This means that amantadine, as other lysosomotropic substances, is trapped by protonation in acidic intracellular compartments and may disturb biochemical processes that require an acidic milieu, such as the proton-driven transport of monoamines into synaptic vesicles. The mean daily oral dose of amantadine is low compared to the high storage capacity of brain and other tissues thus explaining the slow accumulation. Many psychotropic drugs including AD and NL substances also have lysosomotropic properties. A slow accumulation in brain tissue is therefore likely for many AD and NL drugs and has been directly demonstrated for fluoxetine. While lysosomotropism alone is not a sufficient explanation for AD or NL properties of a certain drug, it contributes to high storage capacity and slow accumulation in brain tissue and results in alterations of several biochemical processes.

827.10

DIFFERENT ANXIOLYTIC-LIKE PROFILES OF NOVEL ANTIPSYCHOTICS IN RODENTS. C. Sánchez and J. Arnt*. H. Lundbeck A/S, DK-2500 Copenhagen, Denmark.

An add-on anxiolytic effect is desirable for neuroleptic drugs, as emotional disturbances are frequently accompanying symptoms in schizophrenic patients. The non-classical neuroleptic sertindole shows potent anxiolytic-like effects in rodents and the marmoset[1]. In the present study we have compared the profiles of haloperidol and clozapine with the newer antipsychotics sertindole, risperidone, olanzapine, seroquel and ziprasidone in 3 animal models, i.e. facilitation of exploratory behaviour of rats in a two-compartment black and white box (**B/W**), inhibition of footshock-induced ultrasonic vocalization in adult rats (**USV**) and inhibition of isolation-induced aggressive behaviour in male mice (**AGGR**). Sertindole is the only compound with an anxiolytic-like profile in the **B/W** test (i.e. increased exploration of the white compartment relative to the black). Olanzapine shows an anxiogenic-like profile, and the other compounds are inactive or weakly anxiogenic-like. Clozapine, risperidone, seroquel and olanzapine inhibit **USV**, whereas sertindole and ziprasidone are inactive. Haloperidol is also inactive, even at doses that inhibit locomotor activity markedly. Sertindole, clozapine, risperidone and seroquel inhibit **AGGR**, whereas olanzapine is inactive. Haloperidol is also inactive, even at doses that inhibit locomotor activity of non-aggressive mice markedly. In conclusion, the classical neuroleptic haloperidol is inactive, and the newer antipsychotics and clozapine constitute a heterogenous group with regards to anxiolytic-like effects in rodents.

1. Sánchez C. et al., Drug Devel. Res. **34**, 19.29, 1995

827.11

QUIPAZINE PARTIALLY REVERSED THE RHYTHM SLOWING AND FORCE DECREASING EFFECTS OF CLOZAPINE ON RATS' TONGUE MOVEMENTS DURING LAPPING BEHAVIOR. S. Das* and S.C. Fowler. Dept. of Pharmacology & Toxicology, Univ. of Kansas, Lawrence, KS 66045.

In order to investigate the putative role of serotonin receptor blockade in the slowing of rats' lick rhythm induced by low doses of clozapine (Das & Fowler, Psychopharmacology, 1995), the nonselective serotonin agonist quipazine was administered to rats treated acutely with clozapine. Thirsty rats (n=29) were trained to lap water from a force-sensing disk in 2-min sessions, and Fourier analysis of the resulting force-time recordings provided for the quantification of lick rhythm. By itself, quipazine (0.25, 0.5, 1.0, 2.0 mg/kg, ip. 30 min) significantly quickened the lick rhythm at the lowest 3 doses. Clozapine (1.5, 3.0 mg/kg, ip., 30 min) robustly reduced lick rhythm, and quipazine coadministration partially, but significantly reversed the clozapine-induced slowing. The results were consistent with the hypothesis that serotonin receptors are involved in clozapine's effects on rats' orolingual behavior. Supported by MH43429.

827.12

A SINGLE INJECTION OF IBOGAINE PRODUCES SIGNIFICANT ELEVATIONS IN CORTICOSTERONE LEVELS AND SELECTIVE CHANGES IN THE DOPAMINERGIC SYSTEM IN RAT BRAIN. S.F. Ali*, G.D. Newport, W. Slikker, Jr., R.B. Rothman and M. H. Baumann Neurochemistry Laboratory, Division of Neurotoxicology, NCTR/FDA, Jefferson, AR 72079, and Addiction Research Center, NIDA, Baltimore, MD 21224.

Recently, we reported that a single injection of ibogaine (IBG) produced significant alterations in nitric oxide synthase activity and monoamines levels in mouse brain. Other reports suggest that IBG has moderate binding affinity for the opioid receptors (kappa and sigma). The neurochemical mechanism(s) and its role as a treatment medication for drug addiction is still unclear. The present study was designed to evaluate the effect of IBG on corticosterone levels and the dopaminergic system. Adult SD rats were dosed with 50 mg/kg IBG, ip, and sacrificed 15, 30 minutes 1, 2 and 24 hr later. Trunk blood was collected for hormone levels and brain was dissected for neurochemical analyses. IBG produced significant elevations in corticosterone levels 15 minutes after drug administration that increased with time up to 2 hr and returned to control levels 24 hr after dosing. Dopamine (DA) concentrations in striatum decreased significantly at 30 minutes, 1 and 2 hr after drug administration, however, it returned to control levels at 24 hr. DA metabolites DOPAC and HVA concentration increased in a time dependent manner up to 2 hr. At 24 hr DOPAC concentrations were below control values whereas, HVA returned to control levels. Concentrations of serotonin and its metabolite 5-HIAA were decreased only at 1 hr after the dose administration. These data suggest that a single injection of IBG can produce significant elevations of corticosterone and depletion of DA in a time-dependent manner. Future experiments will determine if these effects are mediated through the kappa or sigma receptors.

827.13

THE PUTATIVE 'ENDABUSE' IBOGAINE INTERACTS WITH THE PCP AND SIGMA BINDING SITES IN RAT BRAIN. Yossef Itzhak* and Syed F. Ali, University of Miami School of Medicine, Miami FL 33101 and Division of Neurotoxicology, NCTR/FDA, Jefferson, AR 72079.

Although the alkaloid ibogaine is a potent hallucinogenic agent some indications suggest that it may be useful for the treatment of opioid and cocaine addiction. The neurochemical mechanism(s) underlying ibogaine effects remain unclear. In the present study we investigated the interaction of ibogaine with the PCP site located in the ionophore of the NMDA receptor complex, with the NMDA receptor binding site, and with sigma binding sites. In well-washed membrane preparations of rat *cortex* and *cerebellum*, the PCP sites were labeled with [^{3}H]MK-801 or [^{3}H]TCP, and the NMDA receptor with [^{3}H]CGP 39653. The sigma-1 and sigma-2 binding sites in rat cortex and cerebellum were labeled with [^{3}H]pentazocine and [^{3}H]DTG, respectively. Results indicated that ibogaine interacts with High and Low affinity PCP binding sites in the cortex: Ki(H) = 0.01-0.05 μM; Ki(L)=2-4 μM, and only with low affinity sites in the cerebellum: Ki=2-4 μM. In contrast, ibogaine (> 100 μM) had no affinity for [^{3}H]CGP 39653 binding sites (cortex and cerebellum). The affinity of ibogaine for sigma-1 and -2 binding sites in cortex and cerebellum ranged from 1.5 - 3 μM. Since NMDA receptor antagonists (e.g., MK-801) are thought to attenuate opiois withdrawal symptoms and cocaine sensitization, it is possible that binding of ibogaine to the PCP sites contributes to its potential 'endabuse' properties. In turn, ibogaine interaction with sigma binding sites may be associated with its adverse effects.

827.14

DANTROLENE DIMINISHED FORELIMB FORCE EMISSION IN A PRESS-WHILE-LICKING BEHAVIORAL TASK. J.A. Stanford* and S.C. Fowler. Dept. of Human Development, Univ. of Kansas, Lawrence, KS 66045.

The peripherally acting striate muscle relaxant, sodium dantrolene, was evaluated as a potential means for modeling reduced muscle tone in freely behaving rats. Animals were trained to use a single forelimb to exert continuous downward pressure on a force-sensing operandum, and water reward was made available to the rat as long as forelimb force was maintained above 20g. Dantrolene (5.0, 7.5, 10.0 mg/kg, ip., 45 min before sessions) significantly and dose-dependently reduced the rats' force output during the hold, but not the initiation segment of forelimb responses. Fourier analysis of the hold segment force-time records indicated that dantrolene diminished power above 5 Hz, but did not slow the oscillatory phenomena quantified by this method. Time on task was not affected by these doses of dantrolene. Together the data suggest that dantrolene can reduce striate muscle force production at doses that have negligible motivational consequences.
Supported by MH43429.

827.15

EFFECTS OF CHRONIC LITHIUM, VALPROIC ACID, AND CARBAMAZEPINE ON THE EXPRESSION OF THE PKC SUBSTRATE MARCKS IN IMMORTALIZED HIPPOCAMPAL CELLS. D.G. Watson[1]*, R.K.McNamara[1], and R.H. Lenox[1,2,3]. Departments of Psychiatry[1], Pharmacology[2], and Neuroscience[3], University of Florida College of Medicine, Gainesville, FL 32610.

Studies in our laboratory and others have provided evidence for a role of PKC in mediating the effects of chronic lithium in the brain (Manji and Lenox, 1994). We have previously reported that chronic lithium alters the expression of a major PKC substrate, MARCKS (**M**yristoylated **A**lanine-**R**ich **C**-**K**inase **S**ubstrate), in both rat hippocampus (Lenox *et al*, 1992), and in immortalized hippocampal cells in culture (Lenox *et al*, 1993). In addition, exposure to phorbol esters also induces a rapid down-regulation of MARCKS protein via a PKC-dependent mechanism (Watson *et al*, 1994). In the present investigation we have compared the effects of chronic exposure to the anti-manic agents lithium, valproic acid, and carbamazepine, on the expression of MARCKS protein in immortalized hippocampal HN33 cells. HN33 cells were grown in inositol-free DMEM media supplemented with 5% fetal bovine serum. Following exposure to lithium chloride (1-10mM), sodium valproate (0.5-1.5mM), and carbamazepine (2.5-100μM), cells were collected, fractionated into soluble and membrane fractions, and MARCKS protein assayed by western blot analysis. Both lithium chloride and sodium valproate exposure produced a significant dose-dependent reduction in MARCKS protein in both the soluble and membrane fractions, following long-term exposure (3-7 days). No alterations in MARCKS protein levels were observed following acute exposure to either agent. In contrast, no changes in MARCKS protein levels were detected following long-term exposure to carbamazepine. Regulation of MARCKS protein may represent a pharmacological property shared by mood stabilizers with therapeutic efficacy in the prophylactic treatment of manic-depressive illness. (Supported by NIMH grant MH50105).

827.16

CPP AND NBQX FAIL TO POTENTIATE DOPAMINE D1 AND D2 AGONIST-INDUCED RESPONSES IN THE 6-OHDA MODEL OF PARKINSON'S DISEASE. A.T. Shropshire* and K.L. Marquis. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

Loschmann et al. (J Neural Transm (1992) [Suppl] 38:55-64) proposed that AMPA antagonists may be useful for the symptomatic treatment of Parkinson's disease. Additionally, Wachtel et al. (Neuroscience Letters, 142 (1992) 179-182) proposed that AMPA antagonists and competetive NMDA antagonists may be suitable as adjuvants for the treatment of Parkinson's disease. This was based on (i) the amelioration of parkinsonian symptomology in the MPTP-treated marmoset upon co-administration of the selective AMPA antagonist NBQX and the competetive NMDA antagonist CPP with threshold doses of l-DOPA, and (ii) the potentiation, by NBQX and CPP, of the turning responses produced by threshold doses of the dopamine agonists, apomorphine and lisuride, in substantia nigra lesioned rats. In the current experiment, we examined the effect of combinations of the dopamine D1/D2 agonist, apomorphine, and the D2 agonist, quinpirole, with different doses of NBQX and CPP on turning behavior in rats with unilateral 6-OHDA lesions of the substantia nigra. Our results fail to support the hypothesis of Loschmann et al. or Wachtel et al. Neither NBQX (0.4 and 1.56 mg/kg ip) nor CPP (0.1 and 0.4 mg/kg ip) potentiated the turning behavior produced by apomorphine (0.05 mg/kg ip or sc) or quinpirole (0.05 mg/kg ip). Both antagonists slightly reduced the dopamine agonist-induced turning in substantia nigra lesioned rats. These results tend to lend support to the findings of Morelli and Di Chiara (Eur J Pharmacol, 182 (1990) 611-612) that blockade of NMDA transmission reduces D2 responses. However, we found no evidence to support their claim that blockade of NMDA transmission potentiates D1 responses in the 6-OHDA model of Parkinson's disease.

827.17

PROGESTERONE METABOLISM IS REGULATED BY CARBAMAZEPINE VIA EFFECTS ON 5α-REDUCTASE IN C6 GLIOMA AND NEUROBLASTOMA CELLS. B.S.Pan*, H.K.Manji and W.Z.Potter, Section of Clinical Pharmacology, ETB, NIMH, NIH, Bethesda, MD 20892

Despite the widespread use of carbamazepine (CBZ) in the treatment of both neurological and psychiatric disorders, its mechanisms of action remain to be elucidated. In recent years, considerable research has demonstrated significant effects of neuroactive steroids on the regulation of neuronal excitability. It is thus noteworthy that we have previously demonstrated that CBZ increases the levels of pregnenolone (a precursor of neuroactive steroids) in a time- and dose-related manner in C6 cells. Although preliminary evidence suggests that these effects may be mediated via peripheral-type benzodiazepine receptors, CBZ has also been postulated to interact with 5α-reductase, the enzyme which converts progesterone to 5α-dihydroprogesterone (DHP); since DHP plays an important role in regulating neuronal activities, we investigated the effect of CBZ on progesterone metabolism in both cultured rat C6 glioma cells and in neuroblastoma cells (since 5α-reductase activity is higher in neurons). We have found that CBZ alters 5α-reductase activity and therefore the ^{3}H-progesterone conversion rate in both C6 cells and neuroblastoma cells. The mechanism(s) by which CBZ regulates 5α-reductase activity, and the potential differences of progesterone conversion in neuronal and glial cells is currently under investigation.

827.18

NON-COMPETITIVE NMDA ANTAGONISTS IMPAIR SPATIAL DELAYED ALTERNATION PERFORMANCE IN RATS: REVERSAL BY ANTIPSYCHOTICS, Anita Verma* and Bita Moghaddam, Department of Psychiatry, Yale University School of Medicine, VA Medical Center 116A/2, West Haven, CT 06516

In the present study, the effect of non-competitive NMDA antagonists on a prefrontal cortex (PFC) sensitive task was examined using a spatial delayed alternation (SDA) paradigm in rats. In addition, *in vivo* microdialysis was used to assess the effect of exposure to ketamine on extracellular dopamine levels in PFC and striatum. The non-competitive NMDA antagonists, ketamine (10, 20 and 30 mg/kg) and MK 801 (0.1 and 0.5 mg/kg), impaired the SDA performance as illustrated by the decrease in the number of correct responses during sessions with 15 sec inter-trial interval, as compared with the corresponding vehicle-treated control group. A significant increase in extracellular levels of dopamine was observed in PFC as compared with striatum following ketamine (30 mg/kg) administration. The disruptive effect of ketamine on SDA performance was reversed by haloperidol (0.1 mg/kg) but not by SCH 23390 (0.1 mg/kg). Raclopride (0.1 and 0.5 mg/kg) produced partial reversal of ketamine-induced impairment of spatial working memory performance. These data suggest impairment of PFC-sensitive SDA performance by non-competitive NMDA antagonists. A modulatory role for dopamine receptor subtypes in ketamine-induced disruption of spatial working memory is also suggested.

828.1

NEUROPHYSIOLOGICAL AND BEHAVIORAL EVALUATION OF TREMBLER MICE BETWEEN 1 AND 2 MONTHS OF AGE. J.A. Gruner* and A.K. Yee. Dept. of Pharmacology, Cephalon, Inc., West Chester, PA 19380.

The Trembler mouse (heterozygous TR-J+, Jackson Laboratories) is considered a model of Charcot-Marie-Tooth (CMT) disease. Both humans with CMT and Trembler mice have homologous mutations in the PMP-22 gene (Suter et al., *TINS 16*:50, 1993). Functional impairment of TR-J+ and wild type littermates was assessed at 37, 51, and 65 days of age to determine whether the relative age of onset and progression of the pathology were similar in mice and humans. Behavioral impairment was assessed by determining the latency for mice to traverse a 60 cm x 19mm rod. Electrophysiological measures included conduction velocities (CV) and amplitudes of sciatic, sural, and caudal nerves. Behavioral and electrophysiological deficits in Trembler mice were apparent at 37 d of age and did not markedly progress (see table; *= $p<0.05$ vs control). Behavioral impairment in TR-J+ mice was indicated by an increased latency to traverse the rod. Conduction velocity deficits for sural and caudal nerves were similar to those shown below for the sciatic n. Amplitude measures for all three nerves were reduced by 68% or more in TR-J+ mice and did not significantly change over time.

Measure	Rod Latency (s)			Sciatic CV (m/s)		
Age	37d	51d	65d	37d	51d	65d
TR-J+	6.5±1.0*	5.2±0.8*	5.5±0.5*	6.2±0.3*	6.9±0.3*	7.8±0.6*
control	3.1±0.6	1.8±0.2	2.0±0.1	24.5±1.8	29.8±0.2	30.1±1.2

In contrast to these data, human neurological symptoms of CMT typically are not seen until late childhood and progress to moderate disability by age 30. We conclude that the Trembler mouse may be an appropriate model for assessing the ability of agents to reverse the neurologic deficits of CMT. However, since neurological deficits in the Trembler mouse appear early and fail to progress, this model is inappropriate for evaluating agents intended to affect the pathogenesis of CMT.

828.2

ALTERED SCHWANN CELL PHENOTYPE ASSOCIATED WITH PMP-22 PRODUCTION IN TREMBLER-J MICE. L. Notterpek, G.J. Snipes* and E.M. Shooter. Depts. of Neurobiol. and Neuropathol., Stanford Univ. Sch. of Med., Stanford, CA 94305

Peripheral myelin protein (PMP-22) is a Schwann cell glycoprotein found in compact myelin of the peripheral nervous system. Mutations, duplications or deletions of the PMP-22 gene are associated with peripheral neuropathies in mouse and human. The Trembler-J (Tr-J) mouse mutant carries the same single point mutation that has been identified in an affected human pedigree. Tr-J animals show severe hypomyelination and reduced steady-state levels of PMP-22 mRNA. The specific aim of our studies is to determine how the Tr-J mutation alters normal processing of PMP-22. Genotyped Tr-J Schwann cells were isolated and cultured in parallel with normal litter mates. Compared to normals, Tr-J Schwann cells exhibit distinct morphology and decreased rate of proliferation when cultured in the presence of forskolin and glial growth factor. Double immunolabeling with polyclonal PMP-22 antisera and monoclonal organelle markers indicate differences in the level and localization of PMP-22 in Tr-J Schwann cells. In normal Schwann cells PMP-22 levels are low and the highest level of immunoreactivity is concentrated in the perinuclear region of the ER. In Tr-J, intense PMP-22 like immunoreactivity extends throughout the cytoplasm, including apparent colocalization with lysosomal structures. These data suggest that increased levels of PMP-22 protein (mutant and wild-type) are associated with decreased proliferation of Tr-J Schwann cells in culture. Studies are in progress to identify further differences in processing and translocation of normal and mutated PMP-22 protein when Schwann cells are cocultured with dorsal root ganglion neurons and induced to myelinate.

828.3

CORTICAL GLIA FROM TS16 TRISOMIC MICE REDUCE CHOLINERGIC EXPRESSION IN CULTURED NEURONS. P.G. Nelson, D.v. Agoston, S.C. Fitzgerald. Lab. Dev. Neurobiol., NICHD, NIH; S. Rapoport*, Z. Galdzicki, Lab. Neurosci., NIA, NIH, Bethesda, MD 20892.

Cultures of mouse ventral spinal cord express choline acetyltransferase (ChAT), detected as enzyme activity in homogenates or immunocytochemically in individual neurons. Glia-neuron interactions important for neuronal survival and cholinergic expression have been demonstrated in this system. We studied cholinergic expression of cultured neurons grown on cerebral cortical glia derived from either normal or TS16 trisomic animals. The TS16 trisomy is of particular interest in that it is homologous to the human Trisomy 21 (Down syndrome). The ChAT enzymatic activity of neurons grown in the TS16 glia was reduced to 54% of control values. Total neuronal counts were no different in the two conditions. TS16 neurons grown on TS16 glia expressed ChAT levels 37% of normal. When TS16 neurons were grown on normal glia, ChAT levels were 63% of normal. This was significantly greater than the values for TS16 neurons growing on TS16 glia. We hypothesize that glial cells derived from TS16 animals are either deficient in some cholinergic trophic agent or, more probably, produce some material with a negative effect on neuronal function with some specificity for cholinergic neurons.

828.4

MONOCYTE RECRUITMENT IN BRAINS OF TRANSGENIC MICE EXPRESSING MONOCYTE CHEMOTACTIC PEPTIDE-1 (MCP-1) IN THE CENTRAL NERVOUS SYSTEM. M.E. Fuentes, M.R. Swerdel, S. Durham, M. Barbacid*, R. Bravo, S. Lira. Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543.

It has been postulated that chemokines mediate cell migration to the central nervous system (CNS) during pathological conditions. Monocyte chemoattractant peptide (MCP-1), a member of the C-C chemokine subfamily, has been shown to have a potent chemoattractant activity for monocytes *in vitro* and to be expressed during the development of experimental immune encephalomyelitis, an animal model of multiple sclerosis. In order to study the biological role of MCP-1 *in vivo*, and to examine its role in the development of brain inflammation, we developed a transgenic model system in which MCP-1 expression is driven by the myelin basic protein promoter. In such transgenic mice, we observed maximal transgene expression (RNA) at three weeks of age throughout the white matter, as expected for this oligodendrocyte-specific promoter. Coincident with the temporal and spatial pattern of transgene expression, we found a significant infiltration of mononuclear cells. The recruited cells were predominantly perivascular in orientation with a modest degree of parenchymal infiltration. The vast majority of the cells recruited were monocytes/ macrophages, according to histological and immuno-histochemical criteria.

These results clearly demonstrate that MCP-1 can direct monocyte trafficking *in vivo*. Furthermore, they establish these transgenic mice as important models to study inflammatory cell recruitment to the CNS and the involvement of chemokines in the ethiopathology of inflammatory brain disease.

828.5

GENOTYPIC INFLUENCES ON CYTOKINES EXPRESSION IN ASTROCYTES. THE ROLE OF THE COMPLEMENT COMPONENT C5. M.G. De Simoni[1], P. Mascarucci[1], T.H. Hogan[2], S.A. Johnson[3*] and G.M. Pasinetti[2]. [1]Mario Negri Research Institute, Via Eritrea 62, Milano, Italy 20157; [2]Division of Neurogerontology, Andrus Gerontology Center, USC, Los Angeles, CA 90089; [3]Cortex Pharmaceutical, Irvine, CA 92718.

Many proinflammatory complement (C) components are associated with amyloid deposits in Alzheimer disease (AD) brain. However, the role of C in AD is little understood and may be pertinent to the recruitment and activation of inflammatory cells. Because astrocytes express C5a receptor (C5aR) and because of the C5 derived anaphylatoxin C5a control of cytokine expression in peripheral cells, we further explored the secretion of TNF and IL-6 in astrocytes of C5 deficient ($C5^-$) mice and compared with astrocytes from congenic C5 sufficient ($C5^+$) mice. We found that monotypic cultures of cortico-hippocampal astrocytes originated from neonatal $C5^-$ mice secrete >8 times more TNF and IL-6 than astrocytes from congenic $C5^+$ mice 24 hrs after endotoxin stimulation (10 μg/ml). No detectable TNF and IL-6 signal was found in conditioned medium of control endotoxin free astrocyte cultures of both genotypes. These data suggest that the lack of C5 (C5a?) might predispose astrocytes to a more reactive state in vitro. These data are consistent with the >2 fold higher induction of the astrocytic markers GFAP and apoE mRNAs in hippocampus of $C5^-$ mice following excitotoxic kainic acid lesions, compared to $C5^+$. These studies give basis for further exploration of the role of recombinant and native C5a anaphylatoxins on cytokine gene expression, glial activation in C5aR knockout mice. This work was supported by the Nathan W. & Margaret T. Shock Aging Research Foundation to GMP.

828.6

GENOMIC IMPRINTING AND AUDIOGENIC SEIZURES IN MICE. M.Banko, K.Allen , S.Dolina, P.Neumann and T.N.Seyfried*Boston College, Boston MA 02167.

Audiogenic seizures (AGS) are severe convulsions induced in some strains of mice by loud, high-frequency sound. AGS susceptibility in DBA/2J (D2) mice is maximum at 21 days of age and gradually subsides with adulthood. Epilepsy prone (EP) mice were selected for high AGS susceptibility in a BALB/c line. Unlike D2 mice, EP mice are maximally AGS susceptible at 30 days of age, and this susceptibility persists into adult ages. The seizure phenotype is similar in both the EP and D2 mice. Crosses were generated between the EP and D2 mice to determine the genetic mechanisms that may account for the difference in seizure susceptibility at 30 days of age. Although EP and D2 mice exhibit high susceptibility to AGS at 30 days of age (86% and 53% seizing, respectively), the reciprocal EPD2F1 and D2EPF1 hybrids were mostly seizure resistant, (14% and 16% seizing, respectively), suggesting that AGS susceptibility in these mice is inherited as a recessive trait. In the EP X EPD2F1 backcross, significant associations were found between AGS susceptibility and several markers near Asp-3 on proximal Chromosome 7. In the reciprocal EPD2F1 X EP backcross, however, no significant associations were found between AGS susceptibility and these markers. These data suggest that genomic imprinting may influence the expression of gene(s) on Chromosome 7 involved with AGS susceptibility. Moreover, these findings indicate that different genetic factors are responsible for AGS susceptibility in the EP and D2 mice, further substantiating interallelic heterogeneity for audiogenic seizure susceptibility in mice. (Supported by NIH grant NS23355, Boston College REG and MRC-Canada)

828.7

PROGRESSIVE COGNITIVE IMPAIRMENT IN DISCRIMINATED AVOIDANCE PERFORMANCE IN GFAP-IL6 TRANSGENIC MICE. L.H. Gold*, C.J. Heyser and I.L. Campbell. Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Transgenic mice that express the pro-inflammatory cytokine IL-6 in the CNS under the regulatory control of the glial fibrillary acidic protein promoter (GFAP) develop progressive neurodegenerative disease. The relationship between the neurological disease exhibited by these mice and functional neurobehavioral outcomes was examined. Heterozygous or homozygous GFAP-IL6 mice and control littermates were repeatedly tested at different ages in a Y-maze task for their ability to learn a nonconditional spatial discrimination to avoid the onset of a mild footshock (5 trials/day for 6 days). Both the number of correct avoidances and errors were used as an index of learning performance. When tested at 3 months of age heterozygous mice were able to learn at the same rate as control mice, whereas homozygous mice were significantly slower to acquire the avoidance response. At 6 months of age, heterozygous mice exhibited a deficit in the ability to learn the avoidance response intermediate to that of the controls and homozygotes. At 12 months of age the performance of both heterozygous and homozygous mice had declined even further. At this age both groups failed to demonstrate acquisition of the avoidance response and made significantly more errors when compared to age-matched controls and their own performance at 3 and 6 months of age. No correlation was observed between motor function/coordination and the impaired learning ability of the transgenic groups and no differences were observed between the groups in a test of shock sensitivity. These data demonstrate a progressive learning disability in the GFAP-IL6 mice which is correlated with the progressive neuropathological changes observed in these animals and is not consequent to impaired motor or somatosensory function.

828.8

EFFECT OF THE WEAVER MUTATION ON THE EXPRESSION OF DOPAMINE TRANSPORTER IN NEURONS OF THE SUBSTANTIA NIGRA PARS COMPACTA. C. Adelbrecht, Y. Agid and R. Raisman-Vozari*. INSERM U 289, 75013 Paris, France.

Under physiological conditions, the reuptake dopamine transporter (DAT), located at the plasma membrane, is responsible for dopamine (DA) transport from the synaptic cleft back into the cytoplasm, where they may be repackaged by the vesicular transporter (VAT) into storage organelles, enabling further removal of DA from the synaptic cleft and protection from degradation. Thus, DA transporters play an important role in neurotransmission and could be targets for pathological and/or pharmacological agents. Although the mechanisms involved in dopaminergic cell loss in the homozygous weaver mice (wv/wv) are still unknown, the earliest and largest defect of the dopaminergic system is the decrease in striatal DA uptake (Simon et al., J. Neurochem. 62,543,1994). The cause of this decrease remains unclear but it could be the consequence of an abnormal expression of uptake- and/or storage-related proteins induced by the weaver mutation. Quantitative in situ hybridization of DAT, VAT and tyrosine hydroxylase (TH) mRNAs, carried out on surviving DA cells of the substantia nigra pars compacta (SNC) from two months old weaver mice (wv/wv) showed a significant decrease of DAT expression (-60%) when compared to normal control mice (+/+). DAT mRNA also decreases in SNC cells in heterozygous weaver mice (wv/+) although differences are not significant. In contrast the expression of VAT and TH mRNAs was not affected in the remaining DA neurons of the weaver mice (wv/wv). The specific decrease of DAT mRNA in the surviving neurons of the adult weaver mice could result from a feed-back regulation due to a deficient interaction between dopaminergic nerve terminals and striatal targets. Nevertheless, a genetic alteration of DAT can not be excluded. Studies of DAT expression at early stage during post-natal development are now underway to answer these questions.

828.9

A NEW SPONTANEOUS MOUSE MUTATION RESEMBLING HUMAN NIEMANN-PICK TYPE C DISEASE. B. Kosaras, A. Li and R.L. Sidman* Div. of Neurogenetics, New England Regional Primate Research Center, Harvard Medical School, Southborough, MA 01772-9102.

Several filial generations after a C57BL/6J x BALB/cByJ cross, a single female mouse in a litter of five was noted at 45 days of age (P45) to show weakness of hindlimbs, tremor, and instability of gait. The motor disorder progressed, the mouse lost weight, and died at P90. Similarly affected mice of both sexes were produced by her sibs, and in subsequent generations in this pedigree. Affected mice have not generated offspring, but the 23/109 affected progeny to date from phenotypically normal carriers strongly suggests autosomal recessive inheritance. At autopsy, the liver color is pink to yellowish-white. Many liver cells are enlarged with multiple vacuoles and granules, some staining with sudan black B for lipids and with the fluorescent filipin reagent for unesterified cholesterol. Macrophages rich in cytoplasmic granules are prominent in spleen, lymph nodes, and lung. Large cells in bone marrow smears showed intense cytoplasmic fluorescence with filipin. Staining of paraffin sections of formalin-fixed tissues with the periodic acid-Schiff method is variable from cell to cell in many organs, but is never strong. Brain weight was about 80% of normal. Somas and dendrites of large neurons in dorsal root ganglia, spinal cord, cerebellum, cerebral cortex and retina were distended with lysosome-sized granules that vary in EM appearance from membrane-bound homogeneous organelles to granules rich in myelin-like membranes. Many myelinated and unmyelinated axons in CNS and PNS are dramatically distended focally to 5–10 times their normal diameter with similar granular inclusions. Cerebellar Purkinje cells are reduced in number. These features are consistent with descriptions by others of mouse and human inherited disorder(s) classified as Niemann-Pick Type C disease. Chemical, genetic and tissue culture tests are in progress.

828.10

CHROMOSOME LOCALIZATION OF THE NEUROLOGICAL MOUSE MUTATIONS TOTTERING (*tg*), PURKINJE CELL DEGENERATION (*pcd*), AND NERVOUS (*nr*). D.B. Campbell* and E.J. Hess. Department of Neuroscience & Anatomy, The Pennsylvania State University College of Medicine, Hershey Medical Center, Hershey, PA 17033.

Tottering (*tg*), Purkinje cell degeneration (*pcd*), and nervous (*nr*) are all recessive neurological mutations in the mouse. The mutated gene has been identified in none of these mutants. Positional cloning allows the identification of mutant genes without *a priori* knowledge of the biochemical function of the gene product or the anatomical site of mutant gene expression. Instead, this cloning method identifies abnormal genes based on their physical location in the genome. However, a major rate-limiting step in positional cloning has been the identification of an exact chromosomal location for the gene of interest. We have developed a strategy to rapidly identify the precise chromosomal regions containing the mutant genes of interest. Our strategy takes advantage of the existence of congenic strains: many mutations in the mouse arose spontaneously in one inbred strain and were subsequently backcrossed onto a different inbred strain. After several generations of backcrossing, the only DNA retained from the original strain should be that immediately surrounding the mutant locus; this DNA is readily distinguishable by simple sequence length polymorphisms (SSLPs) between the original strain and the backcross strain. By screening SSLPs in congenically bred mutant mouse strains, we have pinpointed the chromosomal locations of *tg*, *pcd*, and *nr*. The *tg* mutation lies within the 1.1 centimorgan (cM) interval distal to *D8Mit103* and proximal to *D8Mit79*, *D8Mit105*, and *D8Mit283* on mouse chromosome 8. The *pcd* locus maps to the 3.8 cM region between *D13Mit140* and *D13Mit67* on chromosome 13. The *nr* locus maps between *D8Mit155* and *D8Mit18*, a 5.6 cM interval of chromosome 8. In addition to providing specific map positions for these loci, the tightly-linked molecular markers also allow genotyping of *tg*, *pcd*, and *nr* mutant pups, making feasible developmental studies of the late-onset neurological phenotypes caused by these mutations. Supported by a Klingenstein Fellowship.

828.11

A PRELIMINARY EXAMINATION OF THE ROLE OF LTP/LTD IN AMYGDALA KINDLING USING TRANSGENIC CaMKII-Asp 286 MICE. D.P. Cain* and Mark Mayford. Dept. Psychology/Neuroscience Program, Univ. Western Ontario, London, N6A 5C2 Canada and Howard Hughes Medical Institute/Dept. Neurobiology and Behavior, Columbia Univ., 722 West 168th St., New York, N.Y. 10032.

The role of long term potentiation (LTP) has been hypothesized to be important in the kindling model of epileptogenesis. The role of long term depression (LTD) in kindling is not known. The availability of a line of transgenic mice with a mutant form of calcium calmodulin-dependent protein kinase II, resulting in the production of LTD instead of LTP in response to 5-10 Hz stimulation (Mayford et al., submitted), allows an examination of the role of LTP/LTD in epileptogenesis. Therefore we surgically implanted indwelling electrodes into the amygdala and applied a 1-sec train of pulses once daily to evoke after-discharge (AD), a predominant frequency of which was in the 5-10 Hz range. The duration of evoked AD was nearly twice as long in mutants as in wild-type mice in both the stimulated and contralateral amygdala. Despite this, the rate of kindling to the first generalized convulsion did not differ between the groups (mutants = 12.0 ADs, wild-type = 10.9 ADs). The kindled state was retained equally well by the two groups after a 4-wk stimulation-free interval. These results indicate a partial disconnection between AD duration and rate of kindling development. This suggests that a reduction in LTP associated with epileptiform spiking in the range of 5-10 Hz may be linked with the failure of the mutants to develop kindled seizures more rapidly than wild-type mice. Supported by NSERC and HHMI.

828.12

TARGETING OF THE DOPAMINE D_3 RECEPTOR GENE IN EMBRYONIC STEM CELLS: ENRICHMENT FOR HOMOLOGOUS RECOMBINATION BY DESTABILIZING SELECTION MARKER TRANSCRIPTS FROM RANDOM INTEGRATION SITES. B.V. Skryabin* and C. Schmauss. Dept. Psychiatry & Brookdale Center for Molecular Biology, Mt. Sinai Sch. of Med., New York, NY 10029.

Successful gene targeting by homologous recombination is largely determined by the relative frequency of homologous vs. non-homologous recombination events. A widely used selection method for homologously recombinant ES cells is a positive-negative selection (PNS) which typically involves the placement of the thymidine kinase (TK) gene outside of the targeted homology and the addition of antiviral drugs to the ES cell-selection media. However, the toxicity of such drugs (gancyclovir or FIAU) is thought to impair the ability of ES cells to colonize the germ line. An alternative to the use of antiviral agents in conjunction with G-418 for PNS is to destabilize only that portion of the neomycin phosphotransferase II mRNA that derives from random integration sites (positive selection). We have targeted the dopamine D_3-receptor gene locus in mouse ES cells with 5 different replacement targeting vectors. Four vectors contained one of the following sequence elements that are thought to destabilize the neomycin-resitance-gene (neo^r) transcript: 1.) A poly (A^+) less neo^r-cassette; 2.) a neo^r ribozyme with proven catalytic activity, 3.) a mutant ribozyme placed outside of the targeted homology (control); and 4.) an antisense neo^r sequence. A control vector contained the TK gene placed outside of the targeted homology. Our initial results indicate that these strategies are effective alternatives to the selection with antiviral agents. (Supported by NSF IBN-9409772).

828.13

GENE TRANSFER AND THE EXPRESSION OF AN EXOGENOUS GENE IN VIVO USING THE HEMAGGLUTINATING VIRUS OF JAPAN–LIPOSOME METHOD. K. Kato [a,b], and S. E. Fraser[b,*a] Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara, 630-01, Japan; [b]Division of Biology, Caltech, Pasadena, CA 91125.

We have established a simple and efficient method for gene transfer in vivo in post-mitotic neurons of adult rat brain using nuclear protein, liposomes, and hemagglutinating virus of Japan (HVJ; sendai virus). Simple plasmid DNA and nuclear protein (HMG1: non-histone chromosomal protein high mobility group 1) that co-encapsulated in liposomes were co-introduced into cells by HVJ-mediated membrane fusion. The DNA was then carried into the nuclei of nondividing cells with the aid of the nuclear transport system of HMG1.

HVJ-liposomes containing the *E.coli* β-galactosidase gene under control of a chicken β-actin promoter were stereotaxically injected into various brain areas of adult rats, such as hippocampus, cerebellum, cerebral cortex, or caudate putamen. Neuronal cells that expressed the β-galactosidase gene were detected only in the target area of the injected brain of adult rat for 10 days by light microscopic analysis. Electron microscopic analysis revealed that the product of the histochemical reaction were associated with the nuclear membrane and the endoplasmic reticulum of positive cells; it appeared that the products were translated endogenously. Moreover, the products were observed in typical neuronal cells with large, round, and pale nuclei, and with direct axo-somatic and axo-dendritic synaptic contacts.

We are currently applying this system to investigate rhombomere development in the chicken embryo hindbrain.

828.14

DIRECT cDNA SELECTION BY HYBRIDIZATION OF MOUSE CEREBELLAR LIBRARIES AGAINST MICRODISSECTED DNA POOLS FROM THE MMU16 C3-C4 REGION. J.J. Wei, M.E. Hodes, Y. Wang, Y. Feng, B. Ghetti and S.R. Dlouhy*, Depts. of Pathology and Lab. Medicine and of Med. and Mol. Genetics, Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

The distal one third of mouse chromosome 16 is homologous to a part of human chromosome 21 and contains the murine mutant *weaver* (*wv*). The molecular basis of this disorder remains unknown. In this study, cDNA segments were selected directly by hybridization of newborn mouse cerebellar cDNA against genomic DNA pools generated by microdissection of the MMU16 C3-C4 region. Following two rounds of PCR-based DNA-cDNA hybridization, cDNA fragments were cloned into PCR II. About 15% of 300 recombinants analyzed do not contain repetitive sequence and 75% of these unique sequences were mapped to the microdissected chromosome by analysis of somatic cell hybrids and/or by genetic linkage analysis. More than 60% of such cDNA clones (as assessed by RT-PCR) represent transcripts that are expressed in adult mouse cerebellum and northern blots show that some of these are brain- specific. The cDNAs should be useful reagents for further neuromolecular studies (e.g., as *wv* candidates). One selected cDNA clone appears to represent the glutamate receptor, Glur5, which is specifically expressed in postnatal mouse cerebellum and was mapped previously to MMU16. Seventeen of our cDNAs have been mapped genetically within a 20 cM interval on distal MMU16. We conclude that direct cDNA selection from microdissected materials is a practical way of constructing a transcription map of this region. (Supported by USPHS P01 NS27613 and R01 NS14426)

828.15

CYTOCHROME OXIDASE ACTIVITY IN THE NAPLES HIGH- AND LOW-EXCITABILITY RAT LINES: A STUDY OF BRAIN ACTIVITY DIFFERENCES IN A GENETIC MODEL. K. Nixon*, D. Hu, F. Gonzalez-Lima, and A.G. Sadile[1] Dept. of Psychology and Inst. for Neuroscience, University of Texas at Austin, Austin, TX, 78712, USA; [1]Inst. of Human Physiol., 1st Medical School, University of Naples, Naples, Italy.

Quantitative Cytochrome Oxidase (C.O.) histochemistry, a metabolic marker for neural activity, was used here, for the first time, to detect functional brain differences between three different genotypes. The Naples High-Excitability (NHE) and Naples Low-Excitability (NLE) rat lines were selectively bred according to the frequency of rearings and corner-crossings during forced exposure to spatial novelty, both of which are hallmarks of hippocampal dysfunction. Although several neuro-behavioral studies have shown these animals to be a plausible model for hippocampal function, these results need to be confirmed by other means. Using quantitative image analysis, six areas of interest within the hippocampal formation, including the CA fields, the inner blade, and the outer blade of the dentate gyrus, were sampled in naive rats. Significant differences in C.O. activity were detected between the NHE and random bred controls in the area of the inner blade of the dentate gyrus. Previous research has shown a similar decrement in activity in this same area during high behavioral arousal. These findings support the NHE and NLE rats as models for hippocampal function, and, perhaps, a generalized genetic model of hyperactivity.

828.16

ASSESSMENT OF THYMIDINE KINASE DEFICIENT HERPES SIMPLEX VIRUS TYPE 1 AS A TRANSFER VEHICLE INTO SYMPATHETIC PREGANGLIONIC NEURONS. M.A. LeVatte', G.A. Dekaban and L.C. Weaver, Neurodeg. Research Group, Robarts Research Institute, London, Ontario, N6A 5K8.

The sympathetic nervous system discretely controls blood pressure as well as blood flow to different visceral organs. Long term improvements in faulty control of discrete functional groups of spinal sympathetic preganglionic neurons (SPNs) might be accomplished by introducing corrective genes into these cells using replication-defective herpes simplex virus type 1 (HSV-1). Our initial experiments assessed the suitability of HSV-1 as a transfer vehicle into SPNs. The *Escherichia coli* β-galactosidase (β-gal) gene or the human placental alkaline phosphatase (AP) gene was inserted into the thymidine kinase gene, generating replication-defective TK-β-gal or TK-AP (thymidine kinase mutant of HSV-1 expressing β-gal or AP, respectively). To assess whether these mutants retained their neurotropic properties, TK-β-gal was directly inoculated into the spinal cord of hamsters at thoracic (T) segment 6-8. At 3 or 5 days post-inoculation, neurons and numerous oligodendrocytes in the spinal cord were infected with TK-β-gal. To target only SPNs and to assess uptake and retrograde transport of this replication defective HSV-1 into the cord, the TK-AP was inoculated into the left adrenal gland of hamsters. Two, four or five days later, the spinal cords were removed and examined for the presence of TK-AP using AP histochemistry. At 2 days, many SPNs expressing AP were found in T6-9. Very few oligodendrocytes were infected and few inflammatory infiltrates were visible upon counterstaining the cord. At 4 days, fewer SPNs expressing AP were detectable and infiltrates were present. At 5 days, very few SPNs expressing AP were found and inflammatory infiltrates were more abundant. In more preliminary experiments TK-β-gal also was retrogradely transported from the adrenal gland to the spinal cord. These studies demonstrate that a TK-HSV-1 can be retrogradely transported from the periphery to the spinal cord to transduce limited groups of SPNs specifically. Supported by MRC Canada.

DEVELOPMENTAL DISORDERS III

829.1

QUANTIFICATION AND SPECIFICITY OF HIPPOCAMPAL NEURONAL LOSS FOLLOWING NEONATAL INFECTION WITH LCMV B.D. Pearce*, S.C. Steffensen, S.J. Henriksen, M.J. Buchmeier, J.R. Baldridge, A. Paoletti, A.H. Miller Emory Univ Sch of Med, Atlanta, GA 30322; Scripps Res Inst, La Jolla, CA 92037

While a perinatal viral insult has been implicated as a potential causative factor in developmentally-derived hippocampal pathology, the mechanism by which a viral infection during development could disrupt hippocampal structure and function has not been defined. Accordingly, we infected neonatal rats i.c. with lymphocytic choriomeningitis virus (LCMV) and measured electrophysiological and neuropathological changes in the hippocampus. In rats studied at 84-102 days post infection, virus was cleared from dentate granule neurons, yet, Nissl stained cells in the dentate granule layer were decreased by 67.5% (internal limb) and 74.5% (external limb) in LCMV-infected rats. ($p<0.002$). In addition, in vivo electrophysiological measures, recorded from the hippocampal dentate granule layer in response to paired-pulse stimulation of monosynaptic afferents, revealed a marked decrement in the GABA-mediated recurrent inhibition to dentate granule cells, suggesting that dentate granule cells were receiving enhanced excitatory input, perhaps due to an early loss of GABAergic inhibitory interneurons. A subset of GABAergic interneurons in the dentate gyrus contain parvalbumin which may protect these cells from Ca^{++}-induced damage. Quantification of interneurons stained for parvalbumin revealed a 72% loss of these cells in the dentate gyrus of LCMV-infected rats ($p<0.05$). Since rats were infected prior to the developmental stage at which parvalbumin appears, immature neurons (those lacking their protective parvalbumin) may have been particularly vulnerable to the early effects of the virus. These data suggest a novel neuropathogenic mechanism involving an early virus-induced loss of inhibitory interneurons resulting in an unleashing of excitotoxic synapses on dentate granule cells (with resultant cell death), and thus perpetuation of a pathologic cascade which continues in the absence of detectable virus.

829.2

EFFECT OF CHRONIC HYPOXIA ON CORTICAL CELL NUMBERS AND VASCULAR DENSITY. W.B.Stewart*, M.A. Salvatore, V.V. Pathy, G.G. Haddad, L.R. Ment, and M.L. Schwartz. Depts of Surgery (Anatomy), Pediatrics, Neurology and Neurobiology. Yale Univ. Sch. of Med. New Haven CT 06517.

Premature infants often undergo chronic hypoxic episodes. In order to examine the effects of hypoxia on brain development, we have raised rats from postnatal day 3 to 33 in a chamber where O2 levels were controlled at 9 to 10%. The rats were sacrificed and their brains removed for a quantitative study of cells and microvessels of the neocortex. Cell counts were performed on Nissl stained sections using the optical dissector method. Neurons and glial were counted separately, using nuclear morphology to assign them provisionally to either category. Microvessels were counted using the dissector method in unstained, wet-mounted, randomly oriented cortical sections.

Hypoxic rats (N=5) had smaller cortical volumes than control rats (N=5) (127 mm3 vs 156 mm3, $p<.0.03$). Also, the density of neuronal-like cells was higher in the hypoxic rats (174,000 / mm3 vs 96,000 / mm3, $p<0.02$). Despite the smaller cortical volume, there were more of these cells in hypoxics than controls (22.2 million vs 14.9 million, $p<0.06$). By contrast, the number of glial-like cells were similar (2.6 million - hypoxic vs 2.2 million - control). Hypoxic rats had a higher density of capillaries than controls (518 cap. / mm2 vs 400 cap. / mm2, $p<0.03$).

We conclude that neonatal brain has a number of adaptive responses to hypoxia. These include increased vascular density and alterations in programmed cell death. Definitive analysis of this latter possibility is currently being examined using immunohistochemical methods to discriminate between neurons and glia. In addition, these changes in cortical volume and cell density suggest that there may also be decreases in the volume of the neuropil or the extracellular space. This work was supported by NS32578.

829.3

PRENATAL γ-IRRADIATION REDUCES PREPULSE INHIBITION OF STARTLE RESPONSE IN RATS. M. Mintz, A. Gigi, S. Ben-Eliyahu, & M. Myslobodsky*, Psychobiology Res. Unit, Dept. of Psychology, Tel Aviv Univ., Ramat Aviv, Israel

The pathophysiology of behavioral anomalies caused by prenatal irradiation is frequently obscured by the complexity of behaviors used in such studies. To simplify the experimental models we explored changes in the prepulse inhibition (PPI) in Sprague-Dawley rats submitted to whole body γ-irradiation (Theratron 780 ^{60}Co source at 1.5 Gy and a dose rate of 0.15 Gy/min) on Days 15, 17, or 19 of gestation (G). Sham-irradiated rats served as controls. At postnatal day 25 (P25), startle response was assessed to a train of white-noise bursts (122 dB; 40 msec). Following habituation, rats were exposed to a white-noise prepulse (20 dB; ISI=100 msec). At P25 all irradiated groups had higher startle response to the first noise burst compared to controls ($F_{3,36} = 4.1$, $p<.02$). In G15, startle amplitude remained elevated throughout the whole session. By contrast, other irradiated groups habituated to control level at the same rate. PPI was reduced in all irradiated groups. The retest of startle at P55, showed its facilitation in G17 and G19 groups. In G19, habituation to control levels was markedly delayed ($t_{18}=6.8$, $p<.01$). PPI remained decreased in both irradiated groups (animals irradiated on day 15 were not available for analysis) but not at statistical significance level. The possibility is discused that reduced PPI is associated with deficient telencephalic structures, perhaps the prefrontal cortex and hippocampus that are implicated in PPI of startle response.

829.4

ON THE MECHANISM OF NEONATAL TOLERANCE TO ENERGY DEPRIVATION IN CNS WHITE MATTER. B. R. RANSOM*[1] P. DAVIS & R. FERN[1,2] Dept. of Neurology[1], Yale University School of Medicine, New Haven, and Neuroscience Research Center[2], VA Medical Center, West Haven. CT.

The neonatal CNS is relatively resistant to anoxic injury. Prolonged perinatal anoxia/ischemia, however, causes neurological dysfunction by preferentially damaging white matter. We studied the mechanism of neonatal white matter resistance to anoxia/ischemia using the isolated rat optic nerve, a CNS white matter tract. Optic nerve integrity was monitored electrophysiologically by recording the supramaximal compound action potential (CAP). Neonatal nerves (P6-10) were injured as severely as adult nerves by 60 min of combined anoxia/0-glucose (ischemic conditions). This suggested that adult and neonatal nerves had a similar innate susceptibility to injury from near total energy deprivation (i.e. loss of both aerobic an anaerobic metabolism). Removal of either O_2 or glucose for 60 min, however, produced non-reversible CAP loss in adult but not in neonatal optic nerves. Susceptibility to injury from removal of O_2 or glucose developed rapidly between P10-20. Optic nerves survived if glucose was replaced by lactate, and glycogen contained in astrocytes (none is present in axons) could be a source of lactate in 0-glucose. Block of lactate transport with α-cyano-4-hydroxycinnamic acid potentiated the effect of 0-glucose in both adult and neonatal nerves, which supported this hypothesis. These data suggested that astrocytes protected axons from 0-glucose injury by exporting lactate, presumably following glycogen breakdown, via the extracellular space, to axons. This mechanism could not act to protect neonatal nerves from combined anoxia/0-glucose because lactate metabolism requires the presence of O_2. This mechanism allowed neonatal nerves to survive more effectively than adult nerves in 0-glucose, possibly because of a lower energy requirement in neonatal white matter. Although P6-10 nerves were as prone to injury from combined anoxia/0-glucose as adult nerves, preliminary data indicated that nerves from P1-4 rats were resistant to these conditions. Supported by NIH grants.

829.5

NEUROANATOMICAL ANOMALIES IN MICE EXPOSED TO PRENATAL RADIATION S.L. Schmidt* (1,2), M.H.F. Souza (1), and Y. Abreu-Vilaça (1). (1)Dept Fisiologia, Universidade do Estado do Rio de Janeiro, RJ, 20551030, Brasil. (2)Dept. Psychology, University of Alberta, Canada.

In adult mice, prenatal ionizing irradiation at 16, 17 and 19 days of gestation with 3Gy causes reduction of the corpus callosum (CC). Here, we reported the short-term effects after irradiation at E16 with 3Gy and the effects in adult animals after irradiation at E15 with doses lower than 3 Gy (0.5, 1.5, 1.75, 2.0, 2.5 Gy). To study the short-term effects, offspring of control and irradiated mothers were obtained at E17, E19, PND1 and PND5. The brains were sectioned and stained with either cresyl-violet or Bodien silver-stain. The brains of adult animals irradiated at E15 were cut and stained with cresyl-violet. In normal E17 mice, some cells in the subventricular zone formed a sling across the midline. One day after irradiation, the subventricular zone was disrupted, the sling was not detected and a great number of pyknotic figures was seen in the cortical plate but not in the thalamus (in particular, the dorsal lateral geniculate nucleus seemed normal). At E19, PND1, and PND5, the corpora callosa were reduced or absent in most cases, there was a decrease in cellular density in the cortex, and the thalamus did not present any anomalies. Aberrant fibers were seen after E19 whereas ectopic neurons were found only on PND5. Adult mice irradiated at E15 with 1.5 Gy presented periventricular ectopias and agenesis of the caudal portion of the CC. A dose of 1.75Gy caused CC defects in all cases (75% total callosal agenesis; 25% absence of the caudal portion of the CC), presence of ectopias (100%), a pattern that resembled aberrant fibers, and presence of the longitudinal aberrant bundle (50%). Ectopic neurons in layer I were detected in the 1.75Gy group and were commonly seen in the 2 Gy group. Animals irradiated with doses greater than 2 Gy did not present the pattern that resembled aberrant fibers, but CC defects and periventricular ectopias were consistently found. These data show an association with CC defects and ectopias and suggest that prenatal irradiated mice may be a convenient neuropathologic model of callosal agenesis. In humans, callosal agenesis is usually associated with heteropias and polymicrogyria, often at a frequency higher than can be explained solely by chance.

829.6

INHIBITION OF L1-MEDIATED CELL-CELL ADHESION BY ETHANOL. R. Ramanathan, G. Perides M. E. Charness*, *Dept Neurology, Harvard Medical School, VA Medical Center, West Roxbury, MA 02132.*

Mental retardation, hydrocephalus, and agenesis of the corpus callosum are observed both in fetal alcohol syndrome and in human disorders associated with mutations in the gene for the neural cell adhesion molecule (CAM) L1. We showed previously that ethanol inhibits cell-cell adhesion in neural cells treated with human osteogenic protein-1 (OP-1), a powerful inducer of the genes for L1 and N-CAM (*J. Biol. Chem.* **269**, 9304, 1994). To determine whether ethanol inhibits adhesion mediated by an individual class of immunoglobulin CAMs, we produced stable transfectants of mouse L cells expressing human L1. L cells were transfected with a modified pRc/RSV vector (Invitrogen) containing a cDNA for human L1, selected by growth in G418 medium, and subcloned from single colonies. Stable expression of L1 was demonstrated by Northern blot, Western blot, and immunofluorescence. L1-transfected fibroblasts exhibited increased adhesiveness in a short-term aggregation assay as compared with non-transfected cells and cells transfected with the vector alone. Ethanol (5-50 mM) caused a dose-dependent inhibition of cell-cell adhesion in L1-transfected fibroblasts. The dose response curve for ethanol inhibition of cell-cell adhesion in human L1-transfected fibroblasts was similar to that observed for OP-1 treated neural cells. Ethanol's inhibition of L1-mediated cell-cell interactions could contribute to the nervous system lesions of fetal alcohol syndrome. Moreover, because L1 is required for long-term potentiation (*Nature* **372**, 777 ,1994), inhibition of L1-mediated synaptic plasticity could also play a role in alcohol-associated memory disorders.

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829.7

MEDIAL SEPTAL ATROPHY IN AGED MICE EXPOSED TO A SINGLE DOSE OF ALCOHOL IN UTERO. I. Lee, R. Dumas, M.H. Lee and A. Rabe*. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314

It has been demonstrated that mice exposed to a single of dose of alcohol during neurulation, although while remaining free of gross malformations, show a precipitous deterioration of long-term memory in middle and old age. In order to determine whether the behavioral deterioration is related to anatomical changes in specific brain structures, we performed morphometric analyses in several forebrain structures.

C57Bl/6J mice were exposed on gestation day (GD) 9 to a single oral dose of 5.8 g/kg alcohol (E), which produced approximately 700 mg/dl BAC 1 hour after gavage. Control mice were intubated with isocaloric and isovolumic dextrose solution substituted for alcohol (D). The brains were sampled at 24 months of age. The number of neurons was counted and neuron size was measured in the occipital cortical layers, hippocampus, and medial septum. The only measure yielding a significant group difference was the neuron size in the medial septum: They were significantly smaller in the E than D mice. The diminished neuron size is consistent with the findings that structures along the midline are particularly susceptible to alcohol exposure on GD 9. The involvement of the medial septum in long-term memory has been documented, and the atrophic medial septal neurons in the E mice may account for the premature memory decline reported previously by our laboratory (Dumas and Rabe, 1994).

829.8

ANATOMICAL MALFORMATIONS OF SPINAL CORD AXONAL TRACTS IN A RETINOIC ACID-INDUCED SPINA BIFIDA IN THE RAT EMBRYO. Nathalie Valdés, Stéphane Woerly, Louise Bertrand and Raymond Marchand*. Centre de recherche en neurobiologie, Hôp. de l'Enfant Jésus, Univ. Laval; Canada G1J 1Z4.

Most of the congenital malformations taking place at the level of the spinal cord result from a defective closure of the neural plate. This malformation is commonly refered to as spina bifida. We have developed a model of lumbo-sacral spina bifida with myeloschisis (spina bifida aperta) after administration of retinoic acid (RA). RA induces spina bifida when administred in 3 consecutive forcible feedings each containing 13mg of RA/kg at $E10_0$, $E10_6$ and $E10_{12}$. The objectives of the present work were to study the morphology and organization of the spina bifida (SEM and classical histology) as well as the anatomical organization of the axonal tracts (dextranamine injections) in spina bifida embryos. The detailed study of the descending projections associated to the malformation allowed us to identify defective axonal tracts. For example, a dextranamine injection at a caudal thoracic level shows that some axonal tracts do present an abnormal trajectory at the level of the spina bifida. Axons enter the spina bifida but do not extend beyond it. At the level of the malformation, axons cross the midline and form a U-shaped tract that ascends in the contralateral side. Supported by FRSQ-FCAR-Santé, Canada.

829.9

NEUROBIOLOGICAL MODEL FOR TOURETTE SYNDROME CENTERED ON THE NUCLEUS ACCUMBENS. G.N.O. Brito* and M.N. Azevedo. Setor de Neurociencias, Univ. Fed. Fluminense, Niteroi, Brasil.

Tourette syndrome (TS) is a genetic disorder characterized by chronic multiple motor and vocal tics with a fluctuating course and modulated by internal and external environmental events. TS is more prevalent in males than females, and is commonly associated with behavioral disorders such as obsessive-compulsive disorder and attention deficit hiperactivity disorder. TS symptoms are attenuated by DA antagonists and usually exacerbated by psychostimulants. We propose that dysfunction centered on the nucleus accumbens (NAC) represents the neurobiological basis of TS. NAC is divided into a shell and a core region with distinct anatomical relationships. Additionally, recent evidence indicates that NAC has a micro-organization characterized by modules of distinct neurochemical and neuroanatomical features. These modules could interact via closed parallel and open interconnected circuits as suggested by Joel and Weiner (1994) for the basal ganglia. Our model assumes that events (e.g., maternal stress, steroids, hormones and neurotransmitters) occurring during the development of the nervous system interact with products derived from the expression of the putative gene for TS thereby inducing modular changes in NAC. The clinical presentation, associated behavioral disturbances and response to drugs would depend on the pattern of modular dysfunction.

Supported by CNPq and FUNPENE.

829.10

NONLINEAR DYNAMICS OF RESPIRATORY PATTERNING IN INFANTS WHO SUCCUMB TO THE SUDDEN INFANT DEATH SYNDROME. V.L. Schechtman*, M.Y. Lee, A.J. Wilson, D.P. Southall and R.M. Harper. *Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90095.*

Aberrations in cardiac variability appear in infants who later succumb to the sudden infant death syndrome (SIDS); however, overall respiratory variability in SIDS victims is comparable to that of control infants. We assessed non-linear dynamic characteristics of breath-to-breath intervals collected during sleep in 16 recordings of apparently-healthy infants who subsequently died of SIDS and 35 recordings of age-matched control infants. One-interval/next-interval plots (Poincaré Plots) were used to assess nonstationary aspects of respiratory rate variation. Next-breath dispersion was determined following short, intermediate, and long breath-to-breath intervals, and dispersion at each rate was expressed as a percentage of overall respiratory rate variation. ANOVA was used to identify differences between the SIDS victims and control infants. During quiet sleep, SIDS victims showed increased next-breath dispersion following short respiratory intervals, relative to surviving infants. The findings indicate differences in breath-to-breath patterning of respiration in infants who succumb to SIDS that occur preferentially at high respiratory rates and do not affect overall respiratory variability.

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829.11

SUPPLEMENTARY MOTOR AND PREMOTOR OVERACTIVITY IN STUTTERING: A PET STUDY OF STUTTERING AND INDUCED FLUENCY. P.T. Fox*, R.J. Ingham, J. Costello Ingham, H. Downs, T. Hirsch, J.L. Lancaster Research Imaging Center, University of Texas Health Science Center at San Antonio, TX, 78284-6240

The neural-systems whose dysfunction underlies developmental stuttering are unknown. To address this question, we compared stuttered speech to induced fluency (1) using positron-emission tomography (PET).

Twenty, right-handed men participated: ten developmental stutterers and ten normal controls. In each subject, brain blood flow was imaged 3 times per condition in each of 3 conditions: Solo reading, Chorus reading and Rest. In both Solo and Chorus, subjects read aloud from a passage presented on a video monitor. In Solo, reading was unaccompanied; in Chorus, reading was accompanied by a tape-recording of the same passage. All stutters stuttered frequently during Solo but were stutter-free during Chorus. Controls were stutter-free in both conditions. PET imaging used 15-O, water-bolus methods and Change-Distribution Analysis.

Stuttering elicited strong activations in SMA (medial BA6) and right, superior-lateral premotor cortex (lateral BA6). Chorus reading greatly reduced these abnormal activations. In normal subjects, motor-systems activations in both conditions were similar to those of stutterers during induced fluency. We conclude that developmental stuttering may be caused by excessive neural activity in medial and lateral premotor cortex.

1. Ingham, R. J. & Packman A. J. Speech Hearing Res. 1979, 22: 784-793.

829.12

ANOMALOUS CENTRAL AUDITORY PROCESSING IN MAN.

D. M. Daly*. Box 210855, Dallas, TX 75211.

In processing complex sounds such as in speech people exploit systems common at least to mammals. We report using sets of synthesized sounds[1] to test auditory processing in people with normal pure-tone and speech audiometrics and confirmed history of anomalous auditory processing.

1. An American journalist who could read and write German but was unable to understand or speak it intelligibly classified certain sets anomalously ($p<0.0001$); a daughter fluent in German and tested concurrently performed without difficulty.
2. An 11 yr. boy who had difficulty with spoken but not written material in school classified **gy** sets anomalously; his father, a pilot, performed similarly and reported lifelong difficulties with regional accents (particularly in certain ATC sectors).
3. A mother and daughter classified **gy** and **bw** clearly and similarly, but differently from father and another daughter. Each pair reported the other spoke imprecisely; both pairs differed significantly from normal controls.
4. A father and daughter consistently distinguished as many as 4 classes where normal controls found at most 3. These did not correspond to normal classes nor could normal classes be composed from them.
5. An 11 yr. old boy with articulatory difficulties (palate shape) had normal comprehension; his father had undergone surgery for similar problems as a child. Both performed normally ($1\text{-}p < 0.0001$) over 6 hr. of testing

Cases 1-4 demonstrate a range of stable, anomalous auditory processing which affects ability to understand sounds of a foreign or even native language. Cases 1 and 3 preclude appeal to early environment. Analogous to the anatomic origins in Case 5, Case 4 appears to involve anomalous end organ (possibly basilar membrane) geometry.[2] We believe this method can be used to assess dynamic aspects of auditory functioning as well as central auditory processing.

[1]J Neurophysiol (44:1, 200, 1980); [2]JASA (77:1, 108, 1985) Testing contributed by inventor who retains all proprietary rights and interests.

EPILEPSY: KINDLING

830.1

AMYGDALA-KINDLING LEADS TO LOSS OF GABA-IMMUNOREACTIVE NEURONS IN A TINY AREA OF THE PIRIFORM CORTEX. H. Lehmann, U. Ebert and W. Löscher (SPON: European Neuroscience Association), Dept. of Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine,D-30559 Hannover, Germany

Female Wistar rats weighing 260-280 g were electrically kindled via bipolar electrodes, which were implanted unilaterally into the basolateral amygdala, until 10 fully kindled seizures were elicited. After an unstimulated period of 40 days the rats were sacrificed and a immunohistochemical preparation of brain tissue was performed by using a monoclonal *anti*-GABA antibody. Quantification of GABA-immunoreactive (IR) neurons was carried out in the basolateral amygdala, piriform cortex, dentate gyrus, CA1 and CA3. A kindling ($n=10$) versus a sham-group ($n=6$), which received electrode implantation, but no electrical stimulation, and a naive group ($n=10$) were compared. At the stimulation site i.e. the nucleus of the right basolateral amygdala, a significant loss of GABA-IR neurons was observed in kindled rats that ranged between 31-52%. The extent of cell loss was similar ipsi- and contralateral to the stimulation site. Along the rostro-caudal extension of the piriform cortex a distinct area between +0.2 and -0.8mm from Bregma ipsilateral to the stimulation site showed a significant reduction of GABA-IR cells by 17-26%. In contrast, the hippocampal formation showed no changes in GABA-immunoreactivity. In the sham-group no alterations in any of the above areas were evident. We conclude that amygdala-kindling is associated with permanent GABAergic cell loss the site of stimulation, but also at a circumscribed site of the piriform cortex, which is evidently involved into the phenomenon of kindling.

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830.2

PERSISTENT SPONTANEOUS EPILEPTIFORM DISCHARGES EVOKED BY KINDLING-LIKE STIMULATION IN PIRIFORM CORTEX *IN VITRO*. M. R. Pelletier* and P. L. Carlen, Playfair Neuroscience Unit, Bloorview Epilepsy Research Program, Toronto Hospital, Toronto, ON, M5T 2S8.

Interictal discharges appear first, and with greater frequency, in the piriform cortex after kindling, and this region (layer II) is highly susceptible to neuronal damage after kindling-induced status epilepticus. Brain slices containing the piriform cortex, rostral to the septal pole of the hippocampus, were prepared from Wistar rats. Field potentials were recorded in the endopiriform nucleus with NaCl-filled microelectrodes and evoked via a bipolar stimulating electrode placed inferior to layer II (association fibres). Weak stimulation produced a small amplitude potential (p1). Increasing the stimulation intensity evoked p1 and also a second potential (p2) of greater amplitude and occurring at a latency of 50-100 ms from p1. Increasing further the stimulation intensity decreased progressively the latency of p2. The p2 response was polysynaptic in origin, because only p1 was evoked when stimulation was delivered at 1 Hz. Responses were evoked (0.05 Hz) with the test intensity, which was the threshold intensity required to evoke p2. The slice was tetanized (TET) every 10 min: 100 Hz, 2 sec, twice the test intensity. Stimulation was terminated after the 10th TET and spontaneous discharges were monitored. Spontaneous discharges prior to the first TET were recorded in only 4/14 slices. The frequency of spontaneous discharges increased progressively and persisted for the duration of experiments (longest time recorded 120 min after 10th TET). Evoked responses increased in both amplitude and duration and the threshold to evoke p2 decreased after TET. Bath application of the NMDA antagonist, APV (50 μM; $n=3$), the benzodiazepine agonist, midazolam (1 μM; $n=3$), or the antagonist, flumazenil (500 nM; $n=3$) were without effect on the frequency of spontaneous discharges. This novel model of epilepsy is similar to conventional kindling; decreased threshold to evoke discharges, spontaneous discharges, and persistent.

830.3

IBOTENIC ACID LESIONS OF THE HIPPOCAMPUS REDUCE "WET SHAKES" ASSOCIATED WITH AMYGDALOID KINDLING IN THE RAT. J.L. Meyerhoff*, L.E. Jarrard and D.D. Walczak. Div. Med. Neurosci., Walter Reed Army Institute of Research, Wash., D.C. 20307; Dept. of Psychol., Washington and Lee Univ., Lexington, VA 24450 and Solvay Pharmaceut., Marietta, GA 30062.

"Wet Dog Shakes" (WDS) are associated with kindling of the septum, hippocampus (HC), entorhinal cortex (ERC) or amygdala (Squillace, et al., 1980; Le Gal La Salle et al., 1981; Lerner-Natoli et al, 1984). Injection of colchicine into the HC suppressed by 95% the WDS elicted via ERC kindling (Frush & McNamara, 1986). We evaluated the effect of ibotenic acid (IA) lesions of the HC on WDS elicited by amygdaloid kindling. Bilateral stereotaxic injections of IA into the HC were performed in anesthetized male SD rats and chronic amygdaloid electrodes were placed (Meyerhoff, et al. 1985). IA lesions destroy neurons without affecting fibers of passage. Although lesions did not affect no. of stimulations required to elicit stage 5 seizures, the no. of WDS observed was reduced by 68%. Injection of (TRH) i.c.v. synchronizes HC EEG (Kalivas, 1980) and elicits WDS (Drust & Crawford, 1983). Although other neurohumors might be involved in WDS, kindled seizures increase brain levels of TRH (Meyerhoff, et al, 1990) and HC levels of TRH mRNA (Rosen et al., 1992; Kubek et al., 1993).

830.4

THE EFFECTS OF COMPLETE AND PARTIAL BILATERAL LESIONS OF THE CEREBELLAR DEEP NUCLEI ON AMYGDALOID KINDLING IN THE RAT. G.C. Teskey*, J.K. Min and P.A. Valentine. Behav. Neurosci. Res. Grp., Dept. of Psychology, Univ. of Calgary, Calgary, AB, Canada T2N 1N4.

The role of hindbrain mechanisms involved in the acquisition of electrically induced forebrain seizures has received relatively little attention. While an anti-epileptic influence of the cerebellar fastigial nuclei (CFN) has been documented, conflicting observations have been reported for the cerebellar dentate nuclei (CDN), and interpositus nuclei (CIN). This study was undertaken to facilitate a direct comparison of the role of the cerebellar fastigial, dentate and interpositus nuclei in mediating amygdaloid kindling.

Male, Long-Evans hooded rats were chronically implanted with bipolar electrodes in the amygdala, and one of the CDN, CFN, or CIN were bilaterally electrolytically lesioned. Following a two-week recovery, an afterdischarge threshold was determined. This was achieved by the application of 50 μA stimulation trains consisting of 1.0 msec biphasic square wave pulses at 60 Hz for one second. Failure to elicit a discharge resulted in increasing the current in 50 μA steps until the threshold was surpassed. Daily kindling stimulation was applied at 100-150 μA above threshold until two stage 5 convulsions were elicited. The duration, amplitude, and frequency of evoked afterdischarges (ADs) were measured.

Lesions of the cerebellar deep nuclei influence both the kindling rate and afterdischarge characteristics. Our preliminary results indicate that complete lesions resulted in a reduction in the number of sessions to elicit a stage 5 convulsion, decreased AD durations and an atypical progression through the behavioural stages. Partial lesions were typically associated with an increase in the number of sessions to elicit a stage 5 convulsion and increased AD durations. Supported by NSERC.

830.5

THE EFFECTS OF PICROTOXIN AND ATROPINE ON INTERICTAL SPIKING DURING AMYGDALA KINDLING IN THE GENETICALLY FAST AND SLOW KINDLING RAT STRAINS. J.A. Grabowski* and D.C. McIntyre. Psychology Dept., Carleton Univ., Ottawa, Ont., Canada K1S 5B6.

In this study, two rat strains with naturally different kindling rates and disposition to develop interictal spiking (IIS) were examined. Although IIS is the hallmark of an epileptic focus, the kindling resistant (SLOW) rats showed significantly more IIS during all stages of amygdala kindling than the kindling prone (FAST) rats. In the present study, we examined the IIS, the convulsive seizure (ictal event) and the postictal spike (PIS) for their pharmacological sensitivity in both the FAST and SLOW kindling strains. Rats were amygdala kindled daily at their afterdischarge threshold intensity. Once IIS was established, rats were injected with either drug or saline in a latin square design and the IIS rate was observed for 20 min. Subsequently, the ictal event was triggered, which provoked PISs. The GABA antagonist, picrotoxin (0.5 mg/kg), significantly increased the IIS rate, yet had little effect on the ictal event in both strains, while it slightly depressed the PIS rate in the SLOW rats. Like picrotoxin, the cholinergic antagonist, atropine (25 mg/kg), increased the IIS rate in both strains, but strongly suppressed the ictal event in the SLOW but not the FAST strain, and increased the PIS rate in the SLOW but not the FAST strain. Clearly, both IIS activity and kindling rate show a genetic predisposition, but surprisingly their relationship is inverse. It is also clear that IIS, ictal and PIS events are neither similar in the two strains nor in their sensitivity to pharmacological manipulation.

830.6

AXONAL TRANSPORT VELOCITY CHANGES IN THE AMIGDALINE FIBERS IN THE ANTERIOR COMISSURE BY AMIGDALOID KINDLING. Martinez-Lorenzana G, Talavera E, León-Olea M, Condés-Lara M. and Sitges M*. Dpto. Neurofisiología. Instituto Mexicano de Psiquiatría. Calz. México-Xochimilco No. 101 C.P 14370 México D.F.

Studies have shown that the neuronal activity influences both the uptake and axonal transport processes. Kindling, an experimental model in which the neuronal excitability is enhanced, is achieved following repetitive low intensity subconvulsive electrical stimulations in some cerebral regions, mainly in the limbic system (Goddard et al. Exp. Neurol. 25:295-330.1969). Evidence suggests that the neuronal hyperactivity produced by electrical stimulation involves the following processes: endocytosis, exocytosis, mechanisms related to axonal transport and proteic synthesis. In this study, axonal transport velocity during the neuronal hyperexcitability produced by amygdaloid kindling was investigated using a retrograde tracer such as Wheat Germ Agglutinin coupled to Horseradish Peroxidase (HRP-WGA). The fibers analized were the amigdalae fibers crossing in the anterior commissure. Male Wistar rats were divided into three groups: 1)kindled, 2)Sham and 3)Control. Rats were injected with 10% HRP-WGA (10-20 nl) in the right basolateral amygdaloid nucleus, contralateral to the stimulated amygdala. The survival time was 48 h. Coronal sections comprising the areas from the injection site to the anterior commisure were selected. The HRP-WGA reaction was carried out using TMB according with Mesulam (1982) proceding. The brain sections were analyzed under a light microscope. Our results indicated that the kindled group had an increase in axonal transport of HRP-WGA. This means a greater density of labelled fibers and the localization of labelled neurons at a greater distance from the injection site. The enhanced axonal transport was evident when comparing this group with the sham and control groups. Our results suggest that the increment in neuronal activity, due to full kindling seizures, correlates with an increase in the uptake and transport of HRP-WGA.

830.7

AMYGDALA KINDLING PRODUCES MORE "DEFENSIVE" BEHAVIOR THAN HIPPOCAMPAL OR CAUDATE KINDLING IN RATS. J.P.J. Pinel*, L.E. Kalynchuk, and D. Treit[1]. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C., V6T 1Z4 and [1]Dept. of Psychology, Univ. of Alberta, Edmonton, Alberta, T6G 2E9.

We have previously reported that amygdala-kindling in rats results in elevated levels of several defensive behaviors (Kalynchuk et al., 1995). The purpose of this experiment was to investigate whether this effect would also occur after kindling different brain sites. Bipolar electrodes were implanted in the basolateral amygdala, dorsal hippocampus, or caudate nucleus of 81 male, Long-Evans rats. These rats were divided into six groups: amygdala kindled, amygdala control, hippocampal kindled, hippocampal control, caudate kindled, and caudate control. All rats then received 99 convulsive (kindled) or sham (control) stimulations. One day after its last stimulation, each rat was placed in an open field for 5 minutes and then tested for its resistance to capture from the open field. The following day, each rat was tested on an elevated-plus maze. The site of stimulation had a significant effect on the results of these tests. During the first minute in the open field, the amygdala-kindled rats engaged in less exploration than both the hippocampal or caudate-kindled rats. In addition, the amygdala-kindled rats were more resistant to capture from the open field than the hippocampal or caudate-kindled rats. Finally, the amygdala-kindled rats engaged in more open arm activity and escape behavior from the elevated-plus maze than the hippocampal or caudate kindled rats did. Thus, amygdala kindling results in increased emotionality and defensiveness compared to hippocampal or caudate kindling. This suggests that heightened emotionality in kindled rats may be a consequence of repeated seizure activity in or near the amygdala, rather than the kindled state per se. These findings may be relevant in understanding the fear and affective disturbances often experienced by temporal lobe epileptics. (supported by NSERC grants to D.T. and J.P.J.P., and an M.R.C. scholarship to L.E.K.)

830.8

HIPPOCAMPAL KINDLED SEIZURES DISRUPT PERFORMANCE IN THE MORRIS WATER MAZE. T.H. Gilbert* & M.E. Corcoran. Dept. of Psychology, Univ. of Victoria, POB 3050, Victoria, BC, Canada, V8W 3P5.

The hippocampus is thought to play an important role in the processing of spatial information, in that interference in normal hippocampal functioning can produce deficits in performance of a wide variety of tasks that use spatial navigation for optimal performance. The effects of hippocampal kindling on subsequent learning and memory of a spatial task have been assessed in several studies. Although the studies vary in procedure and site of stimulation, their results fairly consistently suggest that epileptiform activity within the hippocampal formation impairs performance. We have attempted to assess the spatial performance of rats in the Morris water maze task during kindling of hippocampal field CA1.

We used two procedures: (1) seizures were kindled with stimulation of CA1 prior to daily training in the water maze (acquisition); and (2) maze training was imposed until performance stabilized, and then seizures were kindled with stimulation of CA1 prior to daily testing in the maze (retention). In both cases, stimulation of field CA1 was applied 25-45 min prior to daily testing in the maze. Only nonconvulsive afterdischarge (AD) was kindled in some rats in the experimental group, whereas in other rats generalized convulsive seizures were kindled. Yoked controls carried electrodes but did not receive stimulation.

We found that CA1 kindled seizures significantly disrupted water maze performance during both acquisition and retention. Similar effects were produced when either nonconvulsive seizures (AD) or convulsive seizures were evoked. Our findings are consistent with previous results suggesting that kindled epileptiform activity in the hippocampus impairs performance in tasks sensitive to spatial learning and memory. (Supported by NSERC)

830.9

THE EFFECTS OF AMYGDALA KINDLING ON T-MAZE PERFORMANCE IN EPILEPTOGENETICALLY FAST AND SLOW KINDLING RAT STRAINS. W. S. McLeod and D. C. McIntyre*. Depart. of Psychology, Carleton Univ., Ottawa, Canada, K1S 5B6.

Kindling, an animal model of epilepsy, was investgated for having acute and chronic effects on memory. Genetically FAST and SLOW kindling rat strains were tested for working and reference memory in a T-maze before, during and after amygdala kindling. Maze performance was monitored 23 hours after eliciting stage 1 through 5 kindled convulsions, then 1 hour versus 10 minutes after stage 5 convulsions in fully kindled animals. After a seizure free period of 10 days and beyond, maze performance was retested with increasing levels of difficulty in the working memory task. Results indicated that kindled convulsions and kindling *per se*, have acute and chronic effects, respectively, on working memory performance. Working memory was more susceptible to disruption than reference memory. Additionally, the FAST rats were less capable than the SLOW rats on the working memory task before kindling, and showed greater impairment during and after kindling. Discussed are neuropharmacological differences between limbic areas of FAST and SLOW rat strains that may account for the differential sensitivity of working memory to kindling disruption.

830.10

SEIZURE AND SPIKE ACTIVITY INTERRELATIONS DURING RAPID KINDLING IN RABBITS. Olga A. Timofeeva* and Gary M. Peterson. Dept Anatomy & Cell Biology, East Carolina Univ Sch Med, Greenville, NC 27858.

Seizures and interictal spikes are considered to be basic components of epileptic activity, but the relationship between these is poorly understood. The present study examines the relationship between seizure and spike activity using closely-spaced recurrent hippocampal seizures ("rapid kindling"). This model provides an opportunity for examining electrographic epileptic reactions in response to electrical stimuli applied at different stages of seizure susceptibility. Twelve adult rabbits with chronically implanted electrodes were stimulated in the hippocampus once every 5 min to achieve a condition of rapidly recurring seizures. EEG was monitored continuously during the experiment (3-6 h). Electrical stimulations produced progressive seizure development in 8 of the 12 animals. Seizures were induced more frequently with increasing numbers of electrical stimulations. Interictal spiking was observed in 5 of the 8 animals and in all (n=4) rabbits which did not develop seizures. Spiking developed at different stages of kindling. A latency for spike onset following each electrical stimulus train significantly fluctuated due to changes in seizure susceptibility. Two kinds of spike onset latency were distinguished: "short-term" (< 50 s) and "long-term" (> 70 s). "Short-term" spike latency was observed predominantly in 3 situations: in the early phase of a prolonged refractory period (5 min after triggering seizure), when seizure decay occurred, and during an "unsuccessful" kindling procedure. "Long-term" spike latency was observed predominantly in the last phase of a prolonged refractory period (5 min before triggering seizure) and always during the development of ictal events. These data showed that "short-term" latency for spike onset correlated with low susceptibility to seizure whereas "long-term" latency correlated with enhancement of seizure susceptibility. These results suggest that seizure priming and triggering mechanisms temporarily inhibit spike activity.

830.11

Regional Increases in the β Subunit but not the α Subunit of Calmodulin Kinase II mRNA in Kindling. K. Sato[1,2], K. Kashihara[3], K. Morimoto[4] and T. Hayabara[2]. [1]Department of Neuropsychiatry and [3]Department of Neurology, Okayama University Medical School, Okayama 700, Japan. [2]Clinical Research Institute, National Sanatorium Minamiokayama Hospital, Okayama 701-03, Japan. [4]Department of Neuropsychiatry, Kagawa Medical School, Kagawa 761-07, Japan.

Levels of the mRNAs for the α and β subunits of calmodulin (CaM) kinase II were studied in the kindling model of epilepsy. Using in situ hybridization and ^{35}S-labeled oligonucleotide probes, induction of these mRNAs was evaluated in the rat brain just after and 0.5, 1, 2, 4, 8 and 24h after generalized seizures induced by daily electrical stimulations of the amygdala. Four to 24h after the seizures, kindling significantly increased levels of the β subunit of CaM kinase II mRNA in the hippocampus compared with controls which had undergone a sham procedure. Four to 24h after the last stimulation, levels of the β subunit of CaM kinase II mRNA increased significantly by 18 to 28 % in the granule cell layer on each side of the dentate gyrus and by 18 to 30 % in the pyramidal cell layer of the ipsilateral CA2. In the pyramidal cell layer of the ipsilateral CA3, levels increased significantly by 22 % 4h after the seizures. There were no detectable changes in levels of the β subunit of the enzyme mRNA in other areas, which included the amygdala, pyriform cortex, perirhinal cortex and temporal cortex. In contrast, no significant changes in levels of the α subunit of CaM kinase II mRNA were observed in the regions examined after kindling-induced generalized seizures.

These results indicate that the increases in CaM kinase II-dependent protein phosphorylation may be associated with changes in synaptic biochemistry in kindling, and suggest that CaM II kinase may mediate the molecular processes underlying kindling-induced epileptogenesis.

830.12

DIFFERENTIAL EXPRESSION OF GABA AND GLUTAMATE TRANSPORTER SUBTYPES IN FULLY KINDLED ANIMALS T.D. Hernandez*(1), A.E. Kline(1), M. Nakashita(2), H. Obata(2) and N. Saito(2). Department of Psychology(1), Univ. of Colorado, Boulder, CO, 80309 and Laboratory of Molecular Pharmacology(2), Biosignal Research Center, Kobe Univ., Kobe 657, Japan.

Alterations in excitatory and inhibitory neurotransmitter activity have been shown in humans with epilepsy and in animal models of the disorder. In general, there is evidence for upregulation of excitatory transmission coupled with downregulation of inhibitory transmission. While these changes have been noted in terms of receptor function, little is known about seizures and amino acid transporters. Glutamate and GABA are each taken up by specific subtypes of transporters located on pre- or post-synaptic terminals or glia. Alterations in the distribution or concentration of these transporters in an animal model of epilepsy (i.e. electrical kindling) should provide clues as to how the brain responds to recurrent seizure activity. To this end, animals were chronically implanted with a bipolar stimulating electrode in the amygdala and kindled once daily until each responded with a Stage 5 seizure. One week later, animals were sacrificed and prepared for immunocytochemical examination of the cellular distribution of the GABA and glutamate transporter subtypes. The glial type of glutamate transporter (GLT-1) was increased in the kindled side of the piriform cortex, while no significant change was observed in other glutamate transporters. None of the three GABA transporters showed obvious change after kindling. These data will be discussed in the context of how alterations in amino acid transporters might underlie the enhanced seizure susceptibility that is produced via the kindling process.

830.13

AN AUDITORY STIMULUS PRESENTED PRIOR TO AMYGDALA KINDLING ALTERS THE RATE OF SEIZURE PROGRESSION: CONTINUOUS EXPOSURE IS NOT REQUIRED FOR THIS REGION-SPECIFIC EFFECT. A.E. Kline*, V. Revilla, and T.D. Hernandez. Department of Psychology, University of Colorado, Boulder, CO 80309.

Prior work has shown that an antecedent tone presentation during every kindling trial significantly delays the rate of amygdala kindling (Hernandez, Warner, Kline, & Kahler, 1995). The specific aim of this study was, therefore, to alter the presence of the tone and investigate this manipulation on kindled seizure development. To achieve this goal, male Long-Evans rats were implanted with an electrode in the right amygdala and assigned to either a *Tone, No Tone*, or *Tone Discontinued* group and kindled daily. The *Tone* group received the tone on every trial while the *Tone Discontinued* group received it for only the first 5 days and subsequently kindled the same as the *No Tone* group (i.e., not exposed to the tone while receiving the kindling stimulation). The results revealed that prior exposure of the tone significantly delayed Stage 5 seizure development for subjects kindled in the central nucleus and significantly accelerated Stage 5 seizures for those kindled in the amygdalostriatal transition area; this finding was observed whether the tone was presented at each trial or discontinued early during the kindling process. These findings indicate that an auditory stimulus presented prior to amygdala kindling alters (i.e., delays or accelerates) the rate of seizure progression. Furthermore, the alteration is not contingent on continuous exposure to the tone, but does appear to be region-specific. Lastly, the data suggest there is an early critical period during kindling that may be amenable to manipulations. Further research is necessary to determine the nature of these manipulations and what effects they might have on seizure genesis.

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830.14

AUDIOGENIC KINDLING INDUCES INCREASED BURST FIRING RESPONSES TO ACOUSTIC STIMULI IN NEURONS OF THE MEDIAL GENICULATE BODY OF GENETICALLY EPILEPSY-PRONE RATS. P. N'Gouemo* and C. L. Faingold. Dept. Pharmacol. Southern Illinois University School of Medicine, Springfield, IL 62794.

Repetitive audiogenic seizures (AGS) result in expansion of the brainstem neuronal network to forebrain structures, including the medial geniculate body (MGB). We previously observed an increase in acoustically-evoked action potentials (AP) in inferior colliculus (IC) neurons with such an AGS kindling paradigm in genetically epilepsy-prone rat (GEPR-9). The medial part of MGB (MGM) receives input from IC. The present study examined acoustically-evoked MGM neuronal responses in "kindled" GEPR-9 as compared to non-kindled GEPR-9 and normal Sprague-Dawley rats. Each stimulated rat received the electric bell (122 dB SPL) twice daily until the onset of convulsion (GEPR-9 mean latency:14.5 sec) or for 15 sec in control. Each kindled GEPR-9 exhibited 14 AGS. Other GEPR-9s (non-kindled) were used after one AGS. MGM extracellular AP were recorded in anesthetized (ketamine/xylaxine, 85/3mg/kg; i.p.) rats 24h after the last stimulation. Acoustic stimulation consisted of 50 stimuli (½ sec, 12 kHz tone bursts) effective in evoking AGS. Responses were analyzed using poststimulus time histograms (PSTH). Recordings involved 11 neurons in kindled GEPR-9, 10 neurons in non-kindled GEPR-9, and 10 neurons in normals. Over 50% of MGM neurons in the GEPR-9 showed burst firing, as compared to 10% of non-kindled GEPR-9 and normal MGM neurons. The mean number of AP per PSTH of MGM unit bursts was significantly elevated in the kindled GEPR-9 (209±47.5, S.E.M.) at 68 dB SPL in comparison to non-kindled GEPR-9 (97.1±9.7) or control (71.9±19.3). The burst responses in GEPR-9 MGM neurons may be a critical neuronal mechanism of AGS kindling that subserves the increase in AGS severity.(Support NIH NINDS NS 21281)

831.1

PROEPILEPTIC EFFECT OF VALPROIC ACID IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. S. Otoom and K. A. Alkadhi*, Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX 77204-5515.

This study was designed to investigate a proepileptic effect of valproic acid (VPA) in hippocampal slices. Studies were performed on brain slices from male Sprague-Dawley rats using conventional intracellular recordings in hippocampal CA1 pyramidal neurons. Action potentials were evoked in response to brief intracellular current pulses (20 ms, 0.5 nA). Veratridine (0.2-0.3 μM) was used to induce epileptiform discharge in response to these pulses. Large concentrations of VPA (0.5 mM) had a biphasic effect on veratridine-pretreated neurons. VPA initially caused an inhibition of evoked bursting activity but soon the bursting reappeared at a higher frequency. The wash-out of the drug was also biphasic. During the early wash-out of VPA with veratridine-containing artificial cerebrospinal fluid (veratridine-ACSF), a complete inhibition of the bursting was noticed. Prolonged wash-out of VPA restored control-like evoked rhythmic bursting. VPA at concentrations of 2 mM or higher enhanced veratridine-induced bursting and the inhibition phase of the drug was not seen. The wash-out of this VPA concentration was still biphasic, the early wash-out with (veratridine-ACSF) produced inhibition of the epileptiform discharges while prolonged washing restored the rhythmic bursting. Membrane potential and membrane input resistance were measured befor and during the epileptic phase of the drug in the presence of veratridine. VPA (0.5-2 mM) produced a small but non significant decrease in membrane input resistance accompanied by a small (non significant) membrane depolarization in the veratridine-pretreated neurons. These results indicate that VPA in large concentrations have a proepileptic effect in rat hippocampal CA1 pyramidal neurons treated with veratridine.

831.2

THE EFFECTS OF VALPROIC ACID ON PMA-INDUCED REGULATION OF PROTEIN KINASE C ACTIVITY AND NMDA-EPSP IN THE RAT HIPPOCAMPUS. **G. Y.-P. Lee*, T. J. Teyler, and L. M. Brown, Depts. Neuro. and pharm., NE Ohio Univ. Coll. of Med., Rootstown, OH 44272-0095.**

The anti-convulsant drug valproic acid suppressed the NMDA induced [3H]-NE efflux in rat cortical slices (Brown et al., 1994). Chronic incubation of glioma cells with valproic acid (0.6 mM) resulted in decreased PKC activity in both membrane and cytosolic fractions (Chen et al., 1994). Incubation with 1 μM phorbol 12-myristate 13-acetate (PMA) for 10 min resulted in a loss of PKC activity in the cytosolic fraction with a concurrent increase in membrane-associated PKC activity (Watson et al., 1994). We investigate the effect of valproic acid on the PMA-induced translocation of PKC from cytosol to membrane, and the effect of valproic acid on NMDA-EPSP. Brain slices (350 μm) from the hippocampus of adult Long-Evans hooded rats were studied. Control slices were incubated in oxygenated ACSF, and experimental slices were incubated in ACSF containing 100 μg/ml (0.6 mM) valproic acid for 20 min and 1 μM PMA for 10 min. After the treatment, hippocampal slices were washed in PBS, homogenized, and prepared for PKC phosphorylation assay (PKC assay kit, Gibco Co.). 400 μm hippocampal slices were used for extracellular NMDA-EPSP recordings with 10 μM bicuculline and 20 μM DNQX in ACSF. The experimental slices were perfused with valproic acid. Results showed that PMA increased PKC activity in the membrane fraction more than in the cytosolic fraction. Valproic acid decreased this enhancement by PMA, and it also suppressed the population NMDA-EPSP amplitude by 10%.

831.3

EFFECTS OF CARBAMAZEPINE ON $GABA_B$-RECEPTORS: INCREASE OF FIELD POTENTIAL CHANGES. J.v.Wegerer,[1,2] I.S.Roed[3] and J. Walden[1]*; 1:Psychiatr.Univ.-klinik, Hauptstr.5, Freiburg; 2:Inst.f.Biol.III, Schänzlestr.1, Freiburg, 3:Bayer AG, Köln, Germany

Nowadays carbamazepine (CBZ) is established in the treatment of epilepsies as well as of affective and schizoaffective disorders. In former studies of the group calciumantagonistic effects of CBZ were found to be a possible common mechanism of action in both diseases. In this study we investigated a possible interference of the action of CBZ on GABAergic neurotransmission, since an attenuation of GABAergic inhibition is discussed to be involved in both illnesses. Extracellular recordings were carried out from areas CA1 and CA3 of the hippocampal slice (guinea pig). GABA and its subtype agonists were applied by conventional local pressure application technique. CBZ was systemically administered. Results: 1) CBZ produced a small increase in the amplitude of field potential changes (EFP) induced by GABA (9 % after the 6th application during 30 minutes; n = 18). 2) CBZ showed no effect on EFP induced by the $GABA_A$-agonist muscimol (n = 5). 3) CBZ produced an increase in the amplitude of EFP induced by the $GABA_B$-agonist baclofen (18 % after the 6th application during 30 minutes; n = 12). The effects were completely reversible. This may be explained either by a direct action on $GABA_B$-receptors, a positive feedback caused by presynaptic $GABA_B$-autoreceptors or by an interference with postsynaptic intrinsic mechanisms. Further studies must reveal whether this effect contributes to the beneficial effect of CBZ in both diseases.

831.4

EFFECTS OF LEVETIRACETAM (LO59) ON CA3 NEURONS IN THE RAT HIPPOCAMPAL SLICE. S. Birnstiel*, E. Wülfert and S.G. Beck, Dept. Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153.

Levetiracetam is a highly effective novel anticonvulsant with an unknown mechanism of action. To test the hypothesis that levetiracetam exerts its actions by an interaction with gabaergic or glutamatergic neurotransmission, intracellular recordings were obtained from the CA3 pyramidal cell layer of transversal slices of rat hippocampus. Synaptic potentials were evoked by stimulation of the septal pathway. Levetiracetam in a concentration of 10 μM did not significantly alter the membrane potential, input resistance or the amplitude of the normal subthreshold synaptic potential consisting of a fast EPSP and IPSP. In contrast, the area under synaptically evoked epileptiform bursts in the presence of 10 μM bicuculline was decreased by levetiracetam. This inhibition in bursting could be due to an enhancement of slow synaptic inhibitory transmission or a decrease of slow synaptic excitatory transmission by levetiracetam. Levetiracetam (10 μM) did not alter the response to 3-10 μM baclofen. Experiments on a possible interaction of levetiracetam with NMDA- or metabotropic glutamate mechanisms are currently in progress. Supported by UCB Pharma.

831.5

AUTORADIOGRAPHIC STUDY OF FELBAMATE EFFECTS ON $GABA_A$ AND NMDA RECEPTORS.
A. Kume* and R.L. Albin. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48104.

Antiepileptic drug felbamate effects on $GABA_A$ and NMDA receptors were investigated autoradiographically in rat brain. For $GABA_A$ receptor, felbamate produced dose-dependent inhibition of [^{3}H]t-butylbicycloorthobenzoate (TBOB) binding with IC_{50} values approximating 250 μM. Saturation analysis in the presence of felbamate revealed a change in K_d and B_{max}. Dissociation initiated by picrotoxin was accelerated by felbamate. The regional pattern of [^{3}H]TBOB binding inhibition by felbamate was heterogeneous. For NMDA binding, felbamate produced dose-dependent inhibition of [^{3}H]MK-801 binding with the maximal inhibiton of 80% at 1-3 mM and IC_{50} values approximating 800 μM. Saturation analysis in the presence of felbamate revealed a decrease in K_d and B_{max}. The regional pattern of [^{3}H]MK-801 binding inhibition by felbamate was heterogeneous. [^{3}H]glycine binding was not affected by felbamate. High-dose glycine only partially antagonized the felbamate inhibition of [^{3}H]MK-801 binding. Combining our results with reported electrophysiological data suggests that felbamate has effects on both $GABA_A$ and NMDA receptors at clinically relevant concentrations.
Supported by NS19613, AG08671 and Tourette Association Fellowship.

831.6

Actions of Felbamate in the corticostriatal system: beyond antiepileptic effects.
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Clinica Neurologica, Università di Roma Tor Vergata, Rome, Italy
§ Schering Plough, Comazzo, MI, Italy

Felbamate (FBM) is a new anticonvulsant which has been shown to interact with the glycine site of the NMDA receptor. The mechanisms underlying the FBM capability to elevate the seizure threshold and to exert neuroprotective effects are not entirely known. Therefore, we have studied the FBM responses in two brain areas - neocortex and striatum - which are involved in both the seizure propagation and neurodegenerative processes. In particular, we have characterized the effects of FBM on: i) high-voltage-activated (HVA) Ca^{2+} currents in pyramidal and striatal cells, ii) the firing properties of neostriatal neurons, iii) the glutamatergic corticostriatal transmission. Patch-clamp whole-cell recordings on isolated neurons and current-clamp intracellular recordings from brain slices were performed with standard techniques. Our main findings can be summarized as follows:

1) FBM dose-dependently reduced HVA Ca^{2+} currents in either cortical and striatal cells. IC_{50} for this action was respectively 298 nM and 15 M; the FBM modulation of Ca^{2+} conductances was blocked by nifedipine (dihydropyridine-antagonist), not by -conotoxin (blocker of N-type channels),

2) FBM inhibited the high-frequency repetitive firing of striatal neurons; this effect was dependent upon the inactivation of the voltage-dependent sodium current,

3) FBM significantly reduced the cortically driven striatal EPSP only after removal of the magnesium block, therefore suggesting that the NMDA component of the transmission was selectively depressed.

Taken together, these findings strongly support the usefulness of FBM not only as an antiepileptic drug but also as a neuroprotective agent.

831.7

THE EFFECTS OF THE ANTICONVULSANT REMACEMIDE HYDROCHLORIDE AT NEURONAL SODIUM CHANNELS. D.Y. Sanchez* and E.W. Harris. Biology Department, Fisons Pharmaceuticals, PO Box 1710, Rochester, NY 14603.

Whole-cell voltage clamp experiments were performed to measure the effects of remacemide hydrochloride and a metabolite, FPL 12495, on neuronal sodium channels.

Cells were dissociated from rat pups 1 - 8 days of age. A voltage clamp protocol designed to mimic rapid firing of action potentials in the brain was used to measure the use-dependent block of sodium current. Slow inactivation was defined as the amount of inactivation 30ms after the end of the depolarizing pulse. The time course of development of slow inactivation was determined. In addition, slow inactivation was measured following 60s of conditioning at -100 mV and -60 mV.

IV plots showed no effect on activation of sodium channels by either compound. The time course of individual currents in response to depolarizing pulses was not affected by either drug. However, the development of slow inactivation was speeded up and the amount of steady-state slow inactivation was enhanced in the presence of either drug. In addition, both compounds caused an accumulation of sodium channel block with high frequency stimulation. FPL 12495 accumulated block at 30μM whereas remacemide hydrochloride required approximately 100μM for significant accumulation of block.

These findings directly demonstrate that remacemide hydrochloride and its metabolite, FPL 12495, act to block sodium channels. This may explain their ability to suppress epileptic activity.

831.8

EFFECTS INDUCED BY GABAPENTIN ON THE ELECTROPHYSIOLOGICAL PROPERTIES OF BURSTING NEURONS IN THE RAT SUBICULUM IN VITRO. H. Kawasaki, D. Mattia, C. Zona* and M. Avoli, Montreal Neurological Institute, Dept Neurology and Neurosurgery, McGill University, 3801 University St, MONTREAL, QC, Canada H3A 3B4

Sharp-electrode intracellular recordings in current-clamp mode were made from pyramidal-like bursting cells of the rat subiculum in a slice preparation to study the effects of the antiepileptic drug Gabapentin. Gabapentin (100μM) induced a small hyperpolarization (4.2 ± 3.1, $n = 14$) of the resting membrane potential (RMP in control = -60 ± 4.6 mV) and reduced the ability of these neurons to generate bursts of action potentials during depolarizing current pulses ($n = 6$). Such intrinsic bursting ability is thought to be caused by a voltage-gated, Na^+-mediated mechanism (*J Neurophysiol* 70 (1993) 1244-1248). The effect on bursting was also seen when the RMP was brought to control values with intracellular steady injection of depolarizing current. In 11 out of 15 cells, Gabapentin decreased by $16.1 \pm 9.7\%$ the sag that is recorded in subicular neurons during hyperpolarizing current pulses. This sag was reduced and eventually blocked by extracellular application of Cs^{2+}. In spite of this effect, which made hyperpolarizing pulses become larger in amplitude, the tendency to generate rebound bursts was decreased by Gabapentin. The effects of Gabapentin were still observed up to two hours after washout. Our findings indicate that Gabapentin can modify the intrinsic excitability of rat subicular neurons. Some of these effects might be caused by the blockade of an inward current that in current-clamp recordings is apparent during hyperpolarizing current pulses, while the burst depression might be due to an interaction of Gabapentin with voltage-gated sodium channels. Supported by MRC of Canada.

831.9

EFFECT OF GABAPENTIN (GBP) ON AMINO ACIDS (AA) IN RAT HIPPOCAMPAL SLICES *IN VITRO*. I.M. Kapetanovic*[1], C.P. Taylor[2], W.D. Yonekawa[1] and H.J. Kupferberg[1]. [1]Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892 and [2]Dept. Neurosci. Therapeutics, Parke-Davis Research, Ann Arbor, MI 48105.

GBP (1-(aminomethyl)cyclohexaneacetic acid; Neurontin®) is a recently approved anticonvulsant, but its mechanism of action remains unclear. The balance between the activity of inhibitory (GABA) and excitatory (L-glutamate, GLU and L-aspartate, ASP) neurotransmitter AA is important for normal neurological function and may play a crucial role in the pathogenesis and potential treatment of epilepsy. This study examined the effects of GBP on these amino acids as well as L-glutamine (GLN) which is closely related to them by the "GLU-GLN" and "GABA-GLU-GLN" cycles. Because of compartmentation, concentrations of newly synthesized (NEW) AA were examined in addition to the total tissue (BASAL) AA. Isotopic enrichment, after incubation with stable isotopically labeled precursors ($^{13}C_6$-glucose, $^{13}C_2$-GLU, $^{13}C_2$-GABA, or $^{15}N_1$-GLN), was used to measure NEW amino acids. BASAL and NEW amino acids were determined by GC-MS. Due to the known delay in its onset of action, GBP (50 or 100 µM) was added at least 60 min before the incubation with labeled precursors. In some cases, animals were also given a 100 mg/kg i.p. bolus of GBP 1 hr prior to slices. There was no effect of GBP on the uptake of $^{13}C_2$-GABA or $^{13}C_2$-GLU or conversion of $^{13}C_2$-GLU to $^{13}C_1$-GABA. Therefore, these data suggest that GBP does not influence GLU decarboxylase, GLU dehydrogenase or GABA transaminase in this paradigm. However, GBP consistently decreased both BASAL and NEW GLN by about 20-30%. This effect may be due to the inhibition of the system L neutral amino acid transport. In the presence of ^{13}C,^{15}N-leucine, GBP decreased NEW but not BASAL GLU. Previous studies suggest this could be caused by GBP inhibition of system L transport or cytosolic branched-chain amino acid aminotransferase.

831.10

THE EFFECTS OF D-23129 (ADD 230001) AND STANDARD ANTICONVULSANTS ON THE PAIRED-PULSE PARADIGM IN RAT HIPPOCAMPUS *IN VITRO*. W.D. Yonekawa*, I.M. Kapetanovic and H.J. Kupferberg, Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892.

The Antiepileptic Drug Development (ADD) Program is investigating the mechanism(s) of action of clinically used and experimental anticonvulsant drugs using standard electrophysiological techniques. The paired-pulse paradigm consisted of measuring the percent increase of the second CA1 EPSP and population spike (PS) after two consecutive stimulations separated by interstimulus intervals varying between 10 to 200 ms. Results were compared before and after drug addition to the ACSF in a standard submerged slice chamber. The results indicate that the standard anticonvulsant drugs, phenytoin and VPA, had minimal effects on paired-pulse facilitation of both PS and EPSP. The experimental drug, D-20443, the hydrochloride salt of D-23129 (*N*-[2-amino-4-(4-fluorobenzylamino)phenyl]carbamic acid ethyl ester) from AWD GmbH/ASTA Medica AG, Germany, did appear to attenuate the facilitation of the conditioned PS and to a lesser extent, the EPSP slope. The attenuation was greatest at half maximum stimulation. While these data might be indicative of a D-23129 presynaptic effect, the different time profile for EPSP attenuation indicates other mechanisms may be involved. These results do show that it is possible to differentiate among various anticonvulsant drugs using the paired-pulse procedure, permitting speculation on possible mechanisms of action.

TRAUMA: TREATMENT I

832.1

EFFECT OF A OF COMBINATION OF ANTAGONISTS OF NMDA AND NON-NMDA RECEPTORS ON FUNCTIONAL DEFICITS RESULTING FROM SPINAL CORD TRAUMA. Jean R. Wrathall* and Yang Dong Teng, Neurobiology Div., Dept. Cell Biology, Georgetown Univ., Washington, DC 20007.

Ionotropic glutamate receptors play a significant role in secondary injury processes after spinal cord trauma. We have shown that both NBQX, a potent and highly selective antagonist of the AMPA/kainate subtypes of glutamate receptors, and CPP, an antagonist of NMDA receptors, focally administered at 15 min after a standardized traumatic spinal cord injury (SCI), result in an increase in the speed of recovery and reduction in long-term hindlimb deficits. NBQX is also effective when administration is delayed until 4 hrs after SCI. We have now examined the effect of combining CPP administered at 15 min and NBQX at 4 hrs. A weight-drop device was used to produce a standardized incomplete thoracic SCI in rats, and CPP (40 nmoles, 1.68 µl) or vehicle (VEH) alone, administered focally at the injury site at 15 min. At 4 hrs post-injury (p.i.), NBQX (15 nmoles, 1.68 µl), or VEH alone, was similarly injected. Behavioral tests of hindlimb functional deficits were performed at 1 day and weekly thereafter for 9 weeks. The group treated with CPP and NBQX demonstrated a reduction in hindlimb functional deficits compared to VEH-VEH controls that was significant beginning at 2 weeks p.i., as we saw previously with CPP alone. The group receiving VEH-NBQX showed reduced deficits beginning at 3 weeks p.i., as seen previously with NBQX administered at 4 hours However, there was no significant difference in the degree of reduction of long-term deficits between the CPP-NBQX and VEH-NBQX groups from 3 - 9 weeks p.i. These results suggest that there are separate mechanisms involved in the antagonists' effects on speed of recovery from SCI as distinct from their effects on long-term functional deficits. [Supported by NIH-NS28130 and PVA-SCRF # 1232].

832.2

ACTIONS OF SYSTEMIC THEOPHYLLINE ON HEMIDIAPHRAGMATIC RECOVERY IN RATS FOLLOWING CERVICAL SPINAL CORD HEMISECTION. K.D. Nantwi*, A. El-Bohy and H.G. Goshgarian. Department of Anatomy and Cell Biology, Wayne State Univ., Sch. of Med., Detroit, MI 48201

This study assesses the effects of theophylline on functional hemidiaphragmatic recovery after C2 spinal cord hemisection in adult female rats using electrphysiological techniques. Twenty four hours following anesthesia with choral hydrate (400 mg/kg, i.p.) and C2 spinal hemisection, rats were reanesthetized. A tracheostomy and bilateral vagotomy were performed, and the femoral artery and vein cannulated for monitoring blood pressure and drug administration respectively. The phrenic nerve ipsilateral to cord injury was placed on bipolar recording electrodes. The animal was then paralyzed and artificially ventilated. The ventilator was turned off and nerve activity recorded until it ceased. After 30 min, theophylline was administered and the procedure repeated. In another group (spontaneously respiring), both phrenic and diaphragmatic activities were monitored after the drug. In a third group of rats (spontaneously respiring) , activity in both phrenic nerves was recorded simultaneously.

In these experiments, (n=6) theophylline induced biphasic effects; i.e. at a low dose (15 mg/kg) it evoked excitation while at a high dose (30 mg/kg) depression predominated. In the first group of spontaneously respiring animals (n=5), a standard dose of theophylline (15 mg/kg) induced activity in both the phrenic nerve as well as the left hemidiaphragm ipsilateral to the hemisection. In the second spontaneouslyrespiring group (n=9), activity was induced in the nerve ipsilateral to the hemisection and enhanced in the intact phrenic nerve for up to three hours.

These results show for the first time that theophylline can activate a latent motor pathway and thus restore the respiratory drive to phrenic motor neurons which was lost by spinal cord injury. Respiratory activity is not only reestablished in the phrenic nerve made quiscent by hemisection, but it is also enhanced in the contralateral phrenic nerve.

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832.3

EFFECTS OF MODEST SYSTEMIC HYPOTHERMIA IN A RAT MODEL OF CONTUSIVE SPINAL CORD INJURY (SCI). A. Martinez-Arizala*, D.H. Hesse, J. V. Perrone, B.A. Green. The Miami Project, Dept. of Neurology and Dept. of Neurological Surgery, Univ. of Miami School of Med., Miami, FL 33136.

Past studies suggest that hypothermia, in the form of local spinal cord cooling, is beneficial in SCI. These studies required surgical access to the spinal cord and cord temperature (temp) was lowered in the range of 10°C. Recent studies in experimental brain ischemia have shown that modest lowering of brain temp (1-3°C) can significantly lessen the degree of tissue damage. Such decreases may be achieved by using systemic hypothermia, which in contrast to local cord cooling does not require surgical access to the spinal cord. To investigate if modest decreases in temp are also beneficial in SCI, thoracic spinal contusive injuries (T8) of moderate severity were produced in S-D rats using the weight drop technique. Following injury, rats were subjected to systemic hypothermia (n=11; core temp=33°C for four hours), or normothermia (n=9; core temp=37°C for four hours). Following SCI, epidural temp remained one degree higher than core temperature. The mean epidural temp was 34.26 ± 0.16°C (±s.e.m.) and 37.69 ± 0.15°C in the hypothermic and normothermic groups, respectively. Hindlimb Tarlov motor scores one month post-SCI were higher in the hypothermic group, 2.9 ± 0.3, when compared to the normothermic group, 2.4 ± 0.2, although it was not statistically significant (p=0.15). The righting reflex time was significantly better in the hypothermic group; 1.56 ± 0.18 sec compared to 2.8 ± 0.47 sec for the normothermic group (p=0.28). However, inclined plane scores, beam balance performance, and sensory scores were similar in both groups. These results suggest a mild positive trend for this regimen of systemic hypothermia. Detailed histological analysis of the lesions is being used to evaluate the degree of tissue sparing, particularly of the segmental grey matter.

832.4

REDUCTION OF NaCl AMELIORATES ULTRASTRUCTURAL DAMAGE TO DENDROTOMIZED MAMMALIAN SPINAL NEURONS. D. G. Emery*, L. J. Rosenberg and J. H. Lucas. Dept. of Zoology and Genetics, Iowa State Univ., Ames, IA 50011 and Dept. of Physiology, Ohio State Univ., Columbus, OH. 43210

In low Ca^{++} conditions Na^+ and Cl^- influx after dendrotomy causes dilation of the smooth endoplasmic reticulum (SER) and Golgi apparatus in cultured spinal neurons (Exp. Brain Res. 86:60-72). Reducing NaCl by 50% (sucrose substitution) increases neuronal survival after dendrotomy from 28% to 68%, while 100% replacement of NaCl reduced survival to 2% (Neurosci. Abst. 20:353.3). We have examined the ultrastructural damage exhibited by neurons within 30 min. of dendrotomy in serum-free, HEPES buffered medium with 50% or 100% replacement of NaCl by sucrose, in the presence of normal (1.8mM) Ca^{++}. Total replacement of NaCl eliminated Golgi/SER swelling but exacerbated mitochondrial swelling, a putative lethal insult. Replacement of 50% of the NaCl reduced but did not eliminate Golgi/SER swelling but seemed to have no effect on mitochondrial swelling. The sodium component of the injury current may contribute to the elevation of cytosolic free Ca^{++} after dendrotomy by stimulating release of mitochondrial Ca^{++} or imparing the Na^+/Ca^{++} antiporter. Cl^- following Ca^{++} into the SER contributes to osmolysis of this organelle, further disrupting Ca^{++} homeostasis. Interventions which preserve the integrity of the SER may have therapeutic value. The effects of longer survival times and specific Na^+ or Cl^- substitutes on ultrastructural damage and survival after dendrotomy are currently being investigated. Supported by PHS grant NS29683 to JHL

832.5

ULTRASTRUCTURE OF MAMMALIAN SPINAL CORD (SC) NEURONS LESIONED UNDER HYPOTHERMIC CONDITIONS. G. Craenen, D.G. Emery[1], and J.H. Lucas*. Dept of Physiology, The Ohio State University, Columbus, OH 43210. [1]Dept of Zoology, Iowa State University, Ames, IA 50011.

There is considerable interest in the potential of CNS cooling to limit tissue damage during surgery and after traumatic injury. This laboratory has developed a model of nerve cell physical injury in which a UV laser microbeam is used to create defined lesions in murine SC neurons grown in tissue culture (standard injury: transection of a primary dendrite 100 µm from the soma; J. Neurotrauma 4: 231-55, 1985). Studies with this model found that a protocol of carefully controlled cooling (2h at 17°C followed by rewarming to 37°C) after dendrotomy significantly increased neuronal survival (J. Neurotrauma 11: 35-61, 1994). Protection was lost when the period at 17°C was extended. Cooling below 17°C caused a N-methyl-D-aspartate (NMDA) receptor-linked injury.

The present study compared the ultrastructural damage that developed in the perikarya of neurons lesioned at 10°C, 17°C, 27°C and 37°C (8-14 cells/group). D-2-amino-5-phosphonovalerate (10 µM) was added to the medium in all experiments to prevent NMDA-linked hypothermic injury. Cells were fixed 5-25 min after surgery. In general, dilation/vesiculation of the Golgi/endoplasmic reticulum in lesioned neurons decreased as the temperature was reduced. The incidence of cytoplasmic condensation, which may be an early indicator of lethal injury (J. Neurotrauma 4: 231-55, 1985), also decreased with cooling (0% at 10°C, 36% at 17°C, 55% at 27°C and 50% at 37°C). However, somal mitochondria of neurons lesioned at lower temperatures exhibited more stress (lucent matrix foci and/or general dilation) than neurons lesioned at 37°C. A study of the damage that develops in neurons cooled after dendrotomy at 37°C, is in progress. Supported by PHS 29683-04 from NINDS to JHL.

832.6

REDUCTION OF EXTRACELLULAR Na+ INCREASES SURVIVAL OF SPINAL CORD (SC) NEURONS SUBJECTED TO DENDROTOMY. L.J. Rosenberg*, D.G. Emery†, and J.H. Lucas. Dept of Physiology, The Ohio State University, Columbus, OH 43210. †Dept of Zoology, Iowa State University, Ames, IA 50011.

A modified ionic environment (MIE) that limits neuronal deterioration would be useful in situations of trauma or surgery. Previous studies in low Ca+2 MIE's found that Na+ and Cl- contribute to osmolysis of the smooth endoplasmic reticulum (SER) and Golgi in SC neurons subjected to dendrite transection with a UV laser microbeam (Emery et al., Exp. Brain Res. 86, 1991). The SER can provide significant Ca+2 buffering even when intracellular levels exceed 10 µM (Carafoli and Penniston, Sci. Amer. 253, 1985). Survival was significantly higher when neurons were lesioned and maintained for 12h in Hepes buffered, serum-free MIE's with a 50% reduction of NaCl (sucrose substitution, normal Ca+2; Lucas et al., Soc. Neurosci. Abst. 20, 844). The present studies investigated the individual contributions of Na+ and Cl- (normal Ca+2) to neuronal degeneration after dendrotomy.

Study I Na+ substituted MIE's were toxic to unlesioned SC neurons and glia when exposure times exceeded 2h. No visible stress was observed in cultures exposed for 2-12h to Cl- substituted MIE's.

Study II Neurons were lesioned in various MIE's (50-80 cells/group). The normal ionic environment was restored 2h post surgery and survival was evaluated at 24h by erythrosin B.

120 mM NaCl	60 mM NaCl	Na+ substitutes	Cl- substitutes
34%±11	67%±17	Choline 74%±13	Isethionate 30%±12
		*NMDG 63%±17	D-gluconate 32%±12

*NMDG = N-methyl-D-glucamine

Study III of the effects of Na+ and/or Cl- reduction on the ultrastructural damage that develops in lesioned neurons, is in progress. Supported by PHS 29683-04 to JHL.

832.7

COMPARISON OF SCIATIC NERVE GRAFTS AND SYNTHETIC TUBES AS BRIDGES IN SPINAL CORD INJURY REPAIR. J.A. McLane* and J. M. Kerns. Rehabilitation R&D Center, Edward Hines Jr. VA Hospital, Hines, IL 60141 and Department of Anatomy, Rush-Presbyterian St. Lukes Medical Center, Chicago, IL 60612.

Although it has been shown that central nervous system neurons are capable of vigorous attempts at regeneration, functional regeneration is seldom successful because of the formation of a dense scar and the presence of inhibitor factors. There are few exceptions to date in experimental models. Peripheral nerve autografts have been shown to enhance regenerative processes within the optic nerve and other injured central nervous system axons. However, the supply of peripheral nerve to use as autografts is very limited. Therefore, synthetic tubular prostheses are being investigated as a means of enhancing and directing regeneration of neurites in the corticospinal tract of the spinal cord following injury.

In these studies, the corticospinal tracts of rats were cut in the midthoracic region of the spinal cord. At the time of injury one end of an artificial nerve consisting of a 1.2 mm i.d. polysulfone tube (Amicon) filled with 30% Matrigel was implanted at the injury site. The other end of the tube was implanted deep into the spinal cord to approximate the ventral horn at a site caudal to the initial injury. Regeneration of axons within these tubes was compared to regeneration of axons within a similarly grafted segment of fresh or predegenerated, isogeneic peripheral nerve. Comparisons were based upon immunocytochemical and morphometric evaluations.

The synthetic tubes supported a cable of organized cells filling more than 50% of the tube volume. This cable was composed of a mixture of cell types, including fibroblasts, glial cells, and a few bare or loosely ensheathed neurites. The cable was highly vascularized and wrapped by a layer of connective tissue. The nerve grafts contained many neurites which were ensheathed by either Schwann cells or myelin.

Supported by the Rehabilitation R&D Center, Hines VA Hospital.

832.8

PROTECTIVE EFFECT OF GLUTATHIONE GLYCOSIDE IN EXPERIMENTAL SPINAL CORD INJURY. S. Etebar, R. C. Kim*, B. H. Choi. Neuropathology, DVA Med Ctr, Long Beach, CA 90822 and Univ. of Calif., Irvine, CA 92717.

Secondary tissue damage developing subsequent to traumatic or ischemic CNS injury is thought to be related at least in part to the generation of reactive oxygen species (ROS). To determine the effectiveness of the potent antioxidant glutathione (GSH), in comparison to that of methylprednisolone (MP), in protecting against oxidative stress following spinal cord injury, the thoracic spinal cords of Sprague-Dawley rats were compressed by a Sugita aneurysm clip; 15 min later, animals were given either a GSH-glycoside (GSH-gly), a newly synthesized compound in our laboratory, or MP intraperitoneally. Thiobarbituric acid-reactive substances (TBARS) and hydrogen peroxide (H_2O_2) levels were measured in animals sacrificed at 6 hr. Spinal cord TBARS levels were significantly lower in GSH-gly- or MP-treated than in control animals. Tissue H_2O_2 levels were also lower in both treatment groups, although the difference was statistically significant only in those given GSH-gly. In addition, neurological assessment, using the Tarlov grading scale, Rivlin-Tator inclined plane testing and tail-flick response, showed functional recovery to be greater in GSH-gly-treated than in untreated animals. These findings, therefore, suggest that GSH-gly is effective in protecting against ROS-mediated damage following spinal cord injury.

832.9

COMPLEX ENVIRONMENT ATTENUATES SPATIAL MEMORY DEFICITS FOLLOWING CORTICAL CONTUSION. S.A. Baldwin*, T.R. Gibson, R.W. Brown, P.J. Kraemer and S.W. Scheff. Center on Aging and Dept. Psychology, Univ. Kentucky, Lexington, KY 40536.

Exposing weanling rats to a complex environment (CE) can alter brain anatomy by increasing brain weight, cortical thickness, dendritic branching and vasculature. Environmental enrichment has been implicated as an aid in recovery from lesion-induced functional impairments in rats. An animal model for cortical contusion has been developed which manifests many of the neuropathologies seen in human closed head injury. The model involves an electronic controlled pneumatic impact device which can deliver very precise and controlled cortical contusions to an animal. This model can be used to study the effects of environmental enrichment on compensation following injury.

Weanling CE rats were housed together in a large cage filled with novel objects that were changed daily. Isolated condition (IC) rats were kept in standard laboratory cages without the objects. Forty days following CE or IC rearing the rats were anesthetized and subjected to a unilateral cortical injury. Seven days after brain trauma, the animals were tested for spatial memory in a Morris Water Maze. The CE group was significantly better on the spatial memory task. Morphological analysis revealed a significant increase in the cortical thickness for the enriched animals as compared to those in the isolated condition.

While it is unclear at present why the CE group performs better, these animals may have a better General Adaptive Capacity (GAC). The complex environment increases the GAC and thus allows the CE animals to perform better. These animals appear to show a greater compensation ability following the trauma. Supported by NS31220

832.10

MEASUREMENTS OF DELAYED CELL DEATH FOLLOWING CORTICAL CONTUSION INJURY IN RATS.

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Division of Neurosurgery, UCLA Sch. of Med., Los Angeles, CA 90024.

Previous work from our laboratory has demonstrated significant neuronal death (up to 73% cell loss) at 2 weeks following a lateral pneumatic piston injury. There is a sequence of secondary insults including biochemical and metabolic alterations immediately after injury that may increase functional impairment as well as induce cell death. This study utilized cytochrome oxidase histochemistry and cellular cytoarchitecture analysis to examine the time course of pathological changes following a lateral pneumatic piston induced injury in 19 male rats. Under general anesthesia (2.0-2.5 mL/min enflurane; 100% O2) a cortical contusion was produced using a 5 mm diameter flat tip (centered at -3.4 mm from bregma) driven to a depth of 2 mm beneath the cortical surface at a velocity of 1.6 m/s. The development of cavity formation and neuronal necrosis were evaluated immediately, 1 h, 6 h, 1 d, and 3 d following injury. Alternate 20 mm sections were stained with cresyl violet and cytochrome oxidase. Immediate cell death was produced by the mechanical shearing at the rim of the impact site (~20% of impact site) and tearing occurred in the white matter below the site. The cells in the remaining 80% of the impact site have morphological characteristics of viable cells up to 1 d post-injury. This core showed slight depression (80% of contralateral) in cytochrome oxidase staining at 6 h but significant depression (50%) at 1 d and 3 d post-injury. This depression was not present at sites distal from the impact site at any time post-injury. These results indicate a potential relationship between secondary tissue damage and altered physiological states. It suggests a window of optimal effectiveness for pharmacological intervention that will rescue injured cells.
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832.11

AMPHETAMINE ENHANCES METABOLIC RECOVERY FOLLOWING MODERATE AND SEVERE CONCUSSIVE HEAD INJURIES IN ADULT RATS. A. Panigrahy*, S.M. Lee, N. Balady, D.A. Hovda, and D.P. Becker. Division of Neurosurgery, UCLA School of Medicine, LA, CA 90024.

Catecholamine stimulation, via amphetamine (AMPH) administration, has been shown to alleviate the metabolic depression typically seen after brain injury in the rat. Previous work from our laboratory has shown that following a moderate fluid percussion (F-P) brain injury, the local cerebral metabolic rates for glucose (LCMRglc) remain depressed as compared to sham-injured values in several neocortical areas, lasting up to 10 days post-injury. We have also found that increasing the levels of severity of F-P brain injury results in a prolonged metabolic depression. In this study, we assessed whether AMPH administration after F-P brain injury would enhance metabolic recovery from a F-P brain injury, in a dose dependant manner, depending on the level of severity of injury. Fifteen adult male Spraque Dawley rats (250-350g) were F-P injured at moderate or severe injury levels while under general anesthesia. AMPH (2 mg/kg BW) was administered 3h after injury for single treatment experiments and 3,6, and 9 h after injury for multiple treatment studies. After 1 day post-injury, LCMRglc was determined by [^{14}C] 2-deoxy-D-glucose autoradiographic methods. LCMRglc for injured animals without AMPH administration served as control values. Moderate-injured animals (n=3), with a single treatment, exhibited LCMRglc values 13%-32% greater than untreated moderate-injured animals (n=4) in different regions of the neocortex (frontal, parietal, and occipital cortex). Severe-injured animals (n=3), with a single treatment, exhibited LCMRglc values that did not significantly differ from untreated severe-injured animals (n=3) in the cortical regions examined. Severe-injured animals (n=2), with multiple treatments, exhibited LCMRglc values 14-20% greater than untreated severe-injured animals in the cortical regions examined. These results suggest that AMPH administration enhances metabolic recovery in a dose-dependant manner; multiple treatments may be require to enhance metabolic recovery with a more severe injury. Supported by NS30308, Lind Lawrence Foundation, and a Howard Hughes Medical Student Research Training Fellowship.

832.12

KININ B_2 BUT NOT B_1 RECEPTOR ANTAGONIST TREATMENT REVERSES I.C.V. ENDOTOXIN-INDUCED HYPERALGESIA AND FEVER IN THE RAT.

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The pro-inflammatory effects of bradykinin (Bk) B_1 and B_2 receptor stimulation have been extensively studied in models of peripheral inflammation. However all of the components of the kinin system are also present in the CNS (Walker *et al.*, 1995, *Neurochem. Int.*, 26:1-16). The present study examined whether inhibition of kinin activity could also alleviate some effects of CNS inflammation. We have investigated the development of hyperalgesia and fever following i.c.v. injections of *E. Coli* lipopolysaccharide (LPS). Rats received a single i.c.v. injection of LPS (200 ng) under enflurane anaesthesia. Rectal temperature (RT), thermal hyperalgesia and mechanical hyperalgesia were measured before and at 2 h intervals following LPS (n = 6/group). RT, thermal and mechanical hyperalgesia were maximal 6 h after LPS injection. LPS-induced increases in RT, thermal and mechanical hyperalgesia were reversed ($P < 0.05$) by the i.c.v. co-administration of the B_2 receptor antagonist, HOE 140 (10-30 pmol), but not by co-administration of B_1 receptor antagonists, des-Arg^9-Leu^8 Bk (0.1-1 nmol) or des-Arg^{10} HOE 140 (0.1-1 nmol). Systemically administered HOE 140 (0.01-1 µmol/kg, i.v.) produced no significant effect. However LPS-induced fever, thermal and mechanical hyperalgesia were inhibited by either i.c.v. (10 nmol) or i.v. (0.1-1 µmol/kg) administered indomethacin. These results indicate that administration of endotoxin to the CNS induces the development of hyperalgesia and fever and that these responses involve the activity of kinins, via the stimulation of centrally located B_2 receptors, and the formation of prostanoids.

832.13

ADENOSINE PROVIDES PROTECTION FROM CA1 TRAUMATIC NEURONAL INJURY TO HIPPOCAMPAL SLICES. S.J. Stamos*, K.L. Panizzon and R.A. Wallis, Dept. of Neurology, UCLA, Los Angeles, CA 90024 and Sepulveda VAMC, Sepulveda, CA 91343.

Extracellular increases in adenosine concentration during hypoxia-ischemia have been found to be neuroprotective. Several cytotoxic mechanisms mediating hypoxic-ischemic injury have been shown to be active in traumatic neuronal injury as well. Therefore, to assess the possible neuroprotective role of adenosine against traumatic neuronal injury, we investigated whether exposure to adenosine would prevent CA1 neuronal injury from fluid percussion trauma in the hippocampal slice. Treatment with 1.0 mM adenosine begun within one min. after trauma, improved recovery at 95 min. of CA1 orthodromic population spike (PS) from 11% ± 5 to 91% ± 4, and improved recovery of CA1 antidromic PS from 13% ± 2 to 91% ± 3. Tetanic stimulation of 100 Hz for 1 second at supra-maximal threshold given 95 min. following trauma, produced a significant increase in CA1 orthodromic PS of 132% ± 10 over baseline after recovery with adenosine treatment. This increase persisted during one hour of additional monitoring consistent with preserved long-term potentiation. Neuroprotection against trauma was also seen with the A1 receptor agonist, N^6-cyclohexyladenosine (20 µM) which increased CA1 PS orthodromic and antidromic recoveries to 93% ± 2 and 92% ± 3, respectively. These findings indicate that direct adenosine application and more specifically, A1 receptor stimulation, provide protection against CA1 traumatic neuronal injury. This work also suggests that elevations of extracellular adenosine concentration may be a preventive factor against the development of neuronal injury after head trauma. Supported by the VA Research Service.

832.14

P-SELECTIN BLOCKADE AFTER FLUID PERCUSSION INJURY: BEHAVIORAL AND ANATOMIC SEQUELAE. D.O. Maris, R.F. Cody, H. Seckin, S.R. Sharar[1], M.S. Grady*. Depts. of Neurological Surgery and Anesthesiology[1], Univ. of WA Sch. of Med., Seattle, WA 98104.

Ischemia and reperfusion promotes neutrophil migration into brain parenchyma, resulting in free radical formation which is in part responsible for neuronal injury. Traumatic brain injury is frequently associated with ischemia. We examined the effect of blockade of cell adhesion molecules (CAMs; important in neutrophil margination and migration across endothelium)on the anatomic and behavioral sequelae of traumatic brain injury in the rat. Lateral fluid-percussion injury (FPI) was used as the injury model. A standard Morris water maze was used as the behavioral task. A blocking antibody to P-selectin (a CAM important in causing neutrophils to attach to endothelium; Cytel, San Diego) and a non-blocking P-selectin antibody were used as treatments. Thirty-two adult male Sprague-Dawley rats were randomly divided into four groups: injury-only; injured/inert vehicle; injured/CAM antibody; sham injury (all n=8). Rats were given moderate FPI with those rats in the treatment groups immediately receiving an intra-arterial injection. Subjects were "place" tested in the Morris water maze at 7 and 14 days post-injury for spatial learning. Rats were perfused at 14 days post-injury and their brains sectioned. Immunocytochemistry was performed on the forebrain for ChAT and GABA, and on the hippocampus using GFAP, OX-42, OX-6, and ED-1. A separate group of 16 animals underwent identical preparation for myeloperoxidase (MPO) determination in the brain parenchyma.

Rats in the injured-only group performed poorly compared to the sham-injured group ($p < .01$), indicating a spatial learning deficit. Using multiple performance indices, a slight treatment effect was seen for rats in the CAM-blockade group ($p<.05$) in cumulative distance and quadrant time measures; latency values were not significant. No significant differences were observed in any performance index between the control antibody and injury-only groups. Analyses of MPO and anatomical data are currently being conducted. This study suggests that administration of a CAM-blocking agent following injury may attenuate the behavioral effects of TBI. (Supported by NIH P 50 NS30305).

832.15

LIPOSOME-MEDIATED BDNF cDNA TRANSFER IN INTACT AND TRAUMATICALLY INJURED RAT BRAIN. [1]K.Yang*, [1]Y.Iwamoto, [1]X.S.Mu, [1]N.Mu, [1]C.E.Dixon, [1]J.S.Whitson, [2]J.R.Perez-Polo, [1]G.L.Clifton, [1]R.L.Hayes. [1]Neurosurgery, U of TX Health Science Ctr., Houston, TX 77030; [2]Human Biochem. & Genetics, U of TX Medical Branch, Galveston, TX 77555.

The therapeutic potential of various neurotrophins for treatment of the injured central nervous system is widely recognized (Barinaga, et al., Science 264:772-774, 1994). The transient expression produced by liposome-mediated gene transfection may limit its application in diseases caused by genetic defects. However, liposomal transfection of neurotrophin may prove useful for treatment of CNS injury. Our laboratory recently reported reduced neurofilament loss in traumatized septo-hippocampal primary cell cultures by liposome-mediated BDNF cDNA gene transfer (Hayes, et al., Neurosci. Lett. in press; Hayes, et al., this meeting). To further explore the potential of gene therapy in brain injury, we are conducting systematic studies of liposome-mediated neurotrophin gene transfer in uninjured rats and in rats following experimental cortical impact injury. Eight μg of pCMV/BDNF plasmid DNA was mixed with 20μl of commercially available liposomes (DOTMA and DOPE: Gibco-BRL) and either liposomes alone or cDNA/liposomes injected (1.2 μl/min) stereotactically into the right hippocampus of uninjured rats with a microsyringe pump. Rats were sacrificed at various time points up to 6 days following injections. PCR analyses were able to detect BDNF cDNA in hippocampi injected with liposomes complexed with cDNA for BDNF from one to six days after injection. Immunohistochemistry studies indicated that expression of BDNF protein (turkey anti-BDNF: Amgen) extended beyond the injection site. Similar profiles of BDNF gene transfection were seen when liposomes or liposomes/cDNA were injected ten minutes after cortical impact injury. Our results suggest that liposome-mediated transfection of neurotrophin cDNA can be a practical gene transfer method for treatment of brain injury. (Supported by NIH grants PO1 NS31998 and RO1 NS21458)

832.16

LIPOSOME-MEDIATED TRANSFECTION OF NEUROTROPHIN cDNA ENHANCES RECOVERY OF NEUROFILAMENT LOSS AND CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY AFTER INJURY TO SEPTO-HIPPOCAMPAL CELL CULTURES. R.L. Hayes*, K. Yang, J.S. Whitson, W. Le, J.J., Xue, C.E. Dixon, G.L. Clifton, A. Kampfl. Dept. of Neurosurgery, University of Texas Houston Health Science Center, Houston, TX 77030.

Enhancing the availability of neurotrophins following brain injury may have significant therapeutic potential. Thus, we used primary septo-hippocampal cell cultures to study liposome-mediated BDNF and NGF gene transfer following depolarization injury (6.0 min depolarization with 60 mM KCl in the presence of 2.8-5.8 mM Ca^{++}). BDNF or NGF cDNA were subcloned onto a unique Not1 site under the control of the CMV promoter. BDNF or NGF cDNA was complexed with liposomes (1.0-3.0 μl lipofectin:1.0 μg cDNA) and transferred to septo-hippocampal cell cultures one day after neuronal injury. Transfection control cultures were incubated with the equivalent volumes of liposomes without cDNA. Three days after depolarization injury, ChAT activity was determined, or Western blot and immunohistochemical analyses examined losses of neurofilament proteins. In non-transfected cultures, we observed approximately 30% loss of ChAT activity following depolarization injury. Depolarization cultures transfected with NGF cDNA did not manifest any significant ChAT activity loss. Depolarization also produced significant loss of neurofilament proteins in non-transfected cultures. Both Western blot analyses and immunohistochemical studies confirmed that BDNF transfection significantly enhanced the recovery of neurofilament proteins three days following depolarization injury. Liposome-mediated transfection of neurotrophins may be useful for treatment of cytoskeletal derangements and disturbances in cholinergic neurotransmission following central nervous system injury (also see Yang, et al., this meeting). (Supported by NIH grants PO1 NS 31998 and RO1 NS 21458)

832.17

EVOLUTION OF THE ALTERATIONS ON ORAL ACETAMINOPHEN PHARMACOKINETICS INDUCED BY SPINAL CORD INJURY IN THE RAT. P. García-López, G. Castañeda-Hernández, G. Guízar-Sahagún and I. Madrazo*. Proyecto Camina A.C. Calz. Tlalpan 4430, 14050 México, D.F., Mexico.

There is evidence that drug kinetics are altered by spinal cord injury (SCI). Notwithstanding, it is not known if such changes are permanent or evolve during the acute, subacute and chronic phases following SCI. Therefore, we studied acetaminophen kinetics after a single oral 100 mg/kg dose 1, 12 and 50 days after spinal cord contusion at the T8 level in rats. Peak plasma concentration in μg/ml (Cmax), area under the blood concentration against time curve in μg h/ml (AUC) and half-life in h (T1/2) were 115±10, 106±7 and 3.8±0.2 respectively in sham-lesioned control rats. 1 day after SCI Cmax was 54±9*, AUC was 76±7 and T1/2 was 5.6±0.6* (* denotes $p<0.05$ compared to control values), the significant reduction on Cmax indicates a decrease in absorption, whereas the significant longer T1/2 suggests an impaired elimination. As a results, bioavailability, indicated by AUC, did not change significantly. 12 days after SCI Cmax was 77±8*, AUC was 78±7 and T1/2 was 3.9±0.3. This suggests that the effect on elimination was reverted, but not that on absorption. 50 days after SCI Cmax was 133±16, AUC was 129±21 and T1/2 was 4.5±0.3. It appears that, both, absorption and elimination were recovered. It is concluded that SCI results in important pharmacokinetic alterations which, however, revert with time. It then appears that, although dosage regimens should be changed after a recent SCI, fixed regimens are not recommended and hence dosing should be continuously adjusted.

832.18

DELAYED ADMINISTRATION OF AMPHETAMINE IMPROVES SPATIAL LEARNING FUNCTION AFTER LATERAL FLUID PERCUSSION INJURY IN THE RAT. M.R. Prasad,* J. M.Dose, H.S. Dhillon and P. Kraemer. Departments of Surgery and Psychology, University of Kentucky Medical Center, Lexington, Ky 40536.

Several studies indicate that the administration of amphetamine at 24 hr after sensorimotor cortex ablation injury and the practice during drug intoxication facilitate the functional recovery of animals. In the present study, we examined the effects of separate administrations of amphetamine (that can enhance the release of norepinephrine), prazosin (an alpha 1-adrenergic receptor antagonist and methoxamine (an alpha 1-adrenergic receptor agonist) at 24 h after lateral fluid percussion (FP) brain injury on the neurologic motor and cognitive outcome of brain injury. Male Sprague Dawley rats (325-350 g; N=44) animals were pretrained to perform beam walk task. The next day the rats were treated with atropine, anesthetized with sodium pentobarbital (60 mg/kg, IP) and subjected to lateral FP brain injury of moderate severity (2.1-2.2 atm.). At 24 h after injury, after obtaining baseline beam walk scores, animals were injected with saline (N=11) or amphetamine (4 mg/kg, N=12) or prazosin (4 mg/kg, N=11) or methoxamine (10 mg/kg, N=10). None of the above drugs significantly affected the beam walking ability of animals during practice (1 h, 2 h and 6 h after drug treatment) and on days 2 through 10 after brain injury. Amphetamine, but not prazosin and methoxamine, treated animals demonstrated significant ($p<0.05$) improvement in spatial learning ability than those of saline treated animals. These results suggest that amphetamine-improved outcome of FP brain injury may involve a combination of receptors along with alpha 1-adrenergic receptor (Supported by NIH Grant NS 31816).

832.19

MK801 PRETREATMENT NORMALIZES MEMORY DEFICITS BUT NOT ALTERED PHOSPHOINOSITIDE METABOLISM 15 DAYS AFTER MODERATE TRAUMATIC BRAIN INJURY. T.M.DELAHUNTY', J.Y.JIANG, Q-Z. GONG, L.L.PHILLIPS, R.J.HAMM, B.G.LYETH. Division of Neurosurgery, Medical College of Virginia, Richmond, VA 23298.

Traumatic brain injury (TBI) produces significant memory impairment and cognitive deficits at 15 days after injury that are reduced by pretreatment with the NMDA receptor antagonist MK801. We have demonstrated altered muscarinic receptor coupling at 15 days after injury. This study examines the effect of MK801 pretreatment on altered muscarinic receptor coupling. Rats were given (i.p.) a single bolus of MK801 (0.3/mg/kg) and injured 15 minutes later by a 2.0 atmosphere lateral fluid percussion impact. After 15 days survival the rats were sacrificed and hippocampal tissue from injured and sham-injured controls was labelled with [^{3}H]-myoinositol and challenged with 250μM Carbachol. The response of injured animals was compared to that of sham-injured animals. In untreated animals, basal inositol phosphates on the side contralateral to the injury cannula were unchanged by injury but on the side ipsilateral to the injury cannula basal levels were enhanced by 26% ($p=0.01$). The response to carbachol was enhanced by 8% on the contralateral side but diminished by 11% on the ipsilateral side ($p=0.02$). In MK801 pretreated animals basal inositol phosphates were unchanged by injury on the side contralateral to the injury cannula but were enhanced by 46% ($p=0.01$) on the side ipsilateral to the injury cannula. The response to carbachol in treated animals remains enhanced by injury 9% on the contralateral side and diminished by 20% on the ipsilateral side ($p=0.05$). Thus, blockade of NMDA receptors does not protect against alterations in muscarinic responses and exacerbates injury induced changes on the side ipsilateral to the injury cannula. Supported by NIH grants NS 12587 and NS 29995.

832.20

TACRINE SLOWS COGNITIVE RECOVERY AFTER TRAUMATIC BRAIN INJURY AND A MUSCARINIC M2 ANTAGONIST REDUCES ITS EFFECT. B.R. Pike*, R.J. Hamm, D.M. O'Dell, M.D. Temple, and B.G. Lyeth. Dept. of Psychology and Division of Neurosurgery, Virginia Commonwealth Univ./Medical College of Virginia, Richmond, VA 23284-2018.

Posttraumatic neuronal depression is hypothesized to contribute to the long-term cognitive deficits following traumatic brain injury (TBI). Postinjury blockade of the presynaptic muscarinic M2 autoreceptor with BIBN 99 amplifies ACh release and improves cognitive performance following TBI in rats. This investigation tested the effects of tacrine (THA), a cholinesterase (AChE) inhibitor, on cognitive performance following central fluid percussion TBI. In addition, effects of combined administration of BIBN 99 and THA on cognitive performance was also tested. In experiment 1, injured rats (2.1 atm) were injected (i.p.) daily for 15 days with either saline (n=8) or 3.0 mg/kg THA (n=10) beginning 24 h after injury. Cognitive performance was assessed on days 11-15 after injury in the Morris water maze (MWM). ANOVA indicated that there was no difference on MWM latencies over days between Saline and THA treated animals. However, to examine rate of recovery, regression lines were calculated and compared by a t-test. This analysis showed that the THA group recovered at a slower rate than the Saline group ($p<0.01$). In experiment 2, injured rats (2.3 atm) were injected daily with either saline (n=7) or the combination of 0.5 mg/kg BIBN 99 (s.c.) and 3.0 mg/kg THA (i.p.) (n=7) according to protocol of experiment 1. ANOVA indicated that there was no difference on MWM latencies over days between Saline and BIBN 99+THA treated animals. However, a t-test on the regression lines was significant ($p<0.05$) indicating that the combined treated animals recovered at a faster rate than the Saline treated animals. These results suggest that AChE therapy slows cognitive recovery following rodent TBI. Cholinergic amplification with presynaptic M2 muscarinic antagonists appears to be a more efficacious strategy for improving cognitive outcome following TBI. (Supported by NS 12587)

833.1

NERVE GROWTH FACTOR ATTENUATES THE LOSS OF CHOLINERGIC NEURONS IN THE MEDIAL SEPTAL NUCLEUS WHICH OCCURS AFTER FLUID-PERCUSSION BRAIN INJURY IN THE RAT. G. Sinson, E.S. Flamm*, T.K. McIntosh. Division of Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104.

Neurotrophins have potential therapeutic applications in a number of neurodegenerative diseases. Previously we have shown that cortical infusions of NGF can attenuate memory deficits in rodents subjected to lateral fluid-percussion brain injury. This study attempts to establish a histopathologic correlation for these cognitive improvements.

Male Sprague-Dawley rats underwent lateral fluid-percussion brain injury of moderate severity (2.1-2.3 atm.). Twenty-four hours after injury, a mini-osmotic pump was implanted to infuse NGF (n=9) or vehicle (n=5) directly into the region of maximal injury. Infusions continued in all animals for 2 weeks, at which time the pumps were removed. Two weeks later (4 weeks after injury) the animals were sacrificed and compared histologically with uninjured animals (n=5). Measurements of the area (mm2) of the septal nuclei demonstrated significant losses in all injured animals ($p<0.05$). Immunohistochemical identification of cholinergic neurons in the medial septal nucleus also demonstrated a significant loss of these neurons in all injured animals ($p<0.05$). The loss of these cholinergic neurons was significantly less in those injured animals which had received NGF infusion after injury ($p<0.05$). Acetylcholinesterase histochemical staining in the hippocampus was also greater in those animals which had received NGF infusion (but still less than uninjured animals). No differences in the size of the cortical injury were noted.

These data suggest that NGF administration, beginning 24 hours after fluid-percussion brain injury, attenuates the loss of cholinergic neurons in the medial septal nucleus. (Supported, in part, by NIH NS26818 and 08803)

833.2

MCPG TREATMENT OF TRAUMATIC BRAIN INJURY IN THE RAT. Q-Z. Gong, T.M. Delahunty, R.J. Hamm, and B.G. Lyeth*. Division of Neurosurgery, Medical College of Virginia, Richmond, Va 23298-693.

Numerous studies have demonstrated the involvement of ionotropic glutamate receptors in traumatic brain injury (TBI) pathophysiology. This study examined the involvement of metabotropic glutamate receptors (mGluRs) in TBI. Of the seven cloned mGluRs, $mGluR_1$ and $mGluR_5$ are coupled to phospholipase C signal transduction, whereas the other five are negatively coupled to adenylate cyclase. Pharmacological studies have demonstrated that (+)α-methyl-4-carboxyphenylglycine (MCPG) acts as a selective antagonist for $mGluR_1$ and $mGluR_2$, while the (−)MCPG isomer is relatively inactive.

In this study, a single 5.0 μl injection of (+)MCPG (0.02 μM, n=8, 0.2 μM, n=10), (−)MCPG(0.2 μM, n=10) or CSF-vehicle (n=11) was administered into left lateral ventricle 5 minutes prior to TBI. A 2.1 atmosphere fluid percussion pulse was delivered through a 2.6 mm diameter injury cannula centered over the right cortex (bregma -5.0, lateral 3.0 mm). Performance on sensory motor tasks was assessed prior to injury and for 5 days after TBI with beam-balance, beam-walking and rotarod tasks. Memory performance was assessed on days 11-15 after TBI with Morris water maze. TBI produced significant motor and memory deficits in the CSF-treated group. High-dose (+)MCPG-treated rats had significantly smaller deficits compared to CSF-treated rats on beam-walking ($P<0,05$) and memory ($P<0.01$), but did not differ from CSF-treated rats on either systemic arterial blood pressure or heart rate responses to injury. Neither the low-dose (+)MCPG nor (−)MCPG affected behavioral deficits. These results suggest that TBI-induced activation of $mGluR_1$ and/or $mGluR_2$ contributes to TBI morbidity. Blockade of those receptors with (+)MCPG may reduce certain components of TBI morbidity.

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833.3

COMPENSATORY PLASTICITY OF THE BRAIN AFTER TRANSCRANIAL POLARIZATION IN CHILDREN WITH CEREBRAL PALSY. M.Sinyaya*[1], D.Pinchuck , M.Katisheva, E. Sidorenko. [1]Pavlov Institute of Physiology of RAN, Inst. of Exp.Medicine, St.Petersburg, Russia,199034,

Local transcranial polarization was used for correction of motor dysfunctions in children with CP. The state of the brain and spinal cord were examined by analysis of EEG, EMG and habituation. Muscle tonus was recorded by new miotonometer that was developed in the lab. of Ontogenesis in the Inst. of Exp.Med. The clinical effects were depended on scalp location of electrodes, the method (mono or bipolar) of stimulation, strengh of the current and its duration. It was shown that the polarization of the brain has led to the decrease in the variation of EEG, restoration of stability of the interhemispheric connections, formation of normal patterns of EEG and the increase in the role the α-rhythm. We estimated that the muscle tonus, the manipulate test, the habituation process on verbal stimulation, asymmetry of the brain changed after polarization, parallel with the restoration of motor functions. Our methods allow for the control of the rehabilitation process dynamics and for the compensatory ability of the brain and spinal cord to be altered.

833.4

INHIBITORY EFFECT OF NYA ON NOS INDUCTION IN AN ANIMAL MODEL OF CEREBRAL HEMORRHAGE. Z.-C. Peng*[1,3], X.-Q. Li[2], Q.-H. Liang[2], C.-X. Zhu[2], X.-X. Yan[1], X.-Y. Hu[2]. [1]Dept. Anat., [2]Inst. Trad. Chinese & Western Med., Hunan Med. Univ., P.R. China; and [3]Inst. Anat. & Histol., Univ. Verona, Italy.

Nao Yi An (NYA), a complex deriving from Traditional Chinese Medicine, has been found to be effective in the recovery of movement deficits in patients suffering from cerebral hemorrhage. We studied the modification of nitric oxide synthase (NOS) activity in an animal model of cerebral hemorrhage, and the effect of NYA, by means of NADPH-diaphorase (NDP) histochemistry. NYA (4.38 g/kg body weight) was orally administered to adult Wistar rats 2 h before autologous blood injection into the right internal capsule (IC) (T Group rats); NYA administration was then repeated daily until perfusion. NYA was not administered in other animals which received blood injection in the IC (C group). Rats were perfused 2, 4, 7, 14, and 28 days after the operation and brain sections were processed for NDP-histochemistry. NDP-positive non-pyramidal neurons were seen in the cerebral cortex, as normally present. In addition, in the C group of rats, numerous NDP-positive pyramidal neurons and endothelial cells were seen throughout the ipsilateral and contralateral cortex, especially in the operated hemisphere, 2 days after surgery. NDP-positive non-neuronal cells, presumably macrophages and astrocytes, were also seen In the C group in the operated hemisphere, close to the needle track. NDP-positive pyramidal neurons were only seen in the ipsilateral cortex 4 days after the operation, when very few NDP-positive non-neuronal cells were still detectable. NDP-positive non-neuronal cells disappeared afterwards. The number of NDP-positive pyramidal neurons decreased progressively 7 days after the operation and they were not visible after 28 days. Non-neuronal NDP-positive cells were not detected in the T group, in which very few NDP-positive pyramidal neurons were detected only in the area close to the needle track in the earliest stage. Thus, our data indicate that autologous blood injection into the brain may induce NOS expression, and that NYA may inhibit such induction.

833.5

METHYLPREDNISOLONE BLOCKS MEMBRANE LIPID PEROXIDATION BUT DOES NOT SCAVENGE FREE RADICALS. DANXIA LIU* AND LIPING LI. Marine Biomedical Institute and Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77555-1143

Methylprednisolone (MP) reduces impairment in human and experimental animals following traumatic spinal cord injury. To study the mechanisms whereby MP reduces secondary injury, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 0.2 mM in ACSF), a product of membrane lipid degradation, was administered into rat spinal cord through a microdialysis fiber. $OH^{\bullet}$ formation was measured by also administering salicylate (0.5 mM in ACSF) through the fiber as a trapping agent and measuring the products 2,3- and 2,5- dihydroxybenzoic acid by HPLC and electrochemical detection. Malondialdehyde (MDA), an end product of membrane lipid peroxidation, was also measured from dialysates by HPLC and fluorometric detection. We found that $OH^{\bullet}$ and MDA both increased dramatically in response to $PGF_{2\alpha}$ administration. In the MP-treated group, 30 mg/kg and 60 mg/kg body weight of MP completely blocked $OH^{\bullet}$ formation and MDA release respectively. This demonstrates that $PGF_{2\alpha}$ induces $OH^{\bullet}$ formation, thereby causing membrane peroxidation to release MDA and that MP blocks the pathway of $PGF_{2\alpha} \rightarrow OH^{\bullet}$ and subsequent MDA production. Next, we generated $OH^{\bullet}$ by administering Fenton reagents into the rat spinal cord and measured MDA release caused by $OH^{\bullet}$ generation. We found that MDA dramatically increased in response to $OH^{\bullet}$ generation. However, MP (30 mg/kg) had no effect on MDA release. This further demonstrates that MP acts as an antioxident to reduce peroxidative injury, but not as a free radical scavenger. Supported by the Paralyzed Veterans of America Spinal Cord Research Foundation (#1025).

833.6

PROGESTERONE TREATMENT REDUCES GENDER DIFFERENCE IN MORRIS WATER MAZE PERFORMANCE AFTER MEDIAL FRONTAL CORTICAL CONTUSION. R.L. Roof*, J.W. Heyburn, J. Baez, & D.G. Stein. Brain Research Lab, Inst. of Animal Behav., Dept of Psych, Rutgers Univ. Newark NJ 07102.

We previously reported that progesterone (P) reduces cerebral edema associated with cortical contusion in male and female rats[1]. In males this leads to an amelioration of cognitive deficits as well as a reduction of neuronal death in the thalamus[2]. To determine whether the females' injury-induced cognitive deficits are also improved with P treatment, 44 female Sprague-Dawley rats were given medial frontal cortical contusions on the day of proestrus. P or oil injections (4 mg/kg,) were given beginning at 1h after injury, and once each 24h period for 1 week.

Beginning 7 days after surgery, each rat was tested for 10 days in the Morris water maze. Contused females took significantly more time and used a longer path to find the hidden platform compared to sham females. Contused females receiving P injections performed better than females treated with the oil vehicle.

These data were compared to that of male rats previously reported[2]. Sham males and females performed equally well on the MWM, however, after cortical contusion, oil-treated females performed significantly worse than oil-treated males. P treatment reduced this gender difference, and females performed as well as P-treated males.

As with the males, cell density measures demonstrated significant neuronal loss associated with the contusion in the medial dorsal thalamus (MD)as well as in cortical areas adjacent to the contusion area. Reduction of the MD cell loss was seen in P-treated lesion rats. There were no gender differences in cell loss or rescue.

[1]Restor. Neurol. Neurosci. 4, 425-427 (1992). [2]Exp. Neurol. 129, 64-69. (1994). Supported by CDC (R49/CCR208836).

834.1

EFFECT OF IMMUNE PRIMING ON BORNA DISEASE A. J. Lewis[1], J. L. Whitton[2], U. Ngo[3], S. van den Noort*[3] and W. I. Lipkin[3] Depts of Anatomy and Neurobiology[1] and Neurology[3], Univ. of California, Irvine, CA 92717; Scripps Research Institute[2], La Jolla, CA 92037.

Borna disease was first described as an encephalitic disease of horses and sheep in southern Germany. The etiological agent has since been found to be a non-segmented, negative strand RNA virus. Borna disease virus (BDV) causes an immune-mediated neurologic disorder in a wide range of host species. The N protein of BDV, p40, is one of the most abundant viral proteins present in infected cells and tissues and elicits a strong antibody response. A vaccinia virus vector expressing p40 (VVp40) was used to prime an immune response in male Lewis rats prior to challenge with BDV infection. Control animals received either a vaccinia vector expressing an irrelevant antigen or PBS. Six weeks later animals were inoculated intranasally with BDV. Clinical observations were made daily. Animals receiving VVp40 showed intensification of clinical disease when compared with both control groups. At 14, 21, 31 and 36 days post BDV challenge brains were collected for histology and RNA analysis. Examination of inflammation in hematoxylin and eosin stained brain sections showed increased perivascular infiltration. Northern hybridization showed a dramatic decrease in expression of viral RNAs in the experimental group. Immunization with VVp40 resulted in an apparent increase in viral clearance, however encephalitis and clinical symptoms were exacerbated.

834.2

BORNA DISEASE VIRUS-SPECIFIC ANTIBODIES ARE PRESENT IN THE CNS OF INFECTED RATS C. G. Hatalski*[1,3], W. F. Hickey[2], W. I. Lipkin[3] Department of Anatomy & Neurobiology[1], Neurovirology Laboratory[3], Univ. of Cal. Irvine, Irvine, CA 92717; Department of Pathology[2], Dartmouth Hitchcock Medical Center, Lebanon, NH 03756

To study the role of antibodies in the CNS during Borna disease virus (BDV) infection, brain sections from male Lewis rats at different times post-infection (acute 4-6 weeks, chronic 15-25 weeks) were analyzed for detection of IgG. Immunohistochemistry using anti-rat IgG antibodies showed diffuse staining throughout the brain, primarily in gray matter areas and neuropil during both the acute and chronic phases of infection. A few small cells both in the parenchyma and in perivascular cuffs stained intensely; these cells are likely to be plasma cells. Additionally, a few cells with neuronal morphology showed intracellular staining. Western blot analysis was performed to compare the levels of IgG in rat brains from the acute and the chronic phase of infection. The intensity of staining with anti-rat IgG in Western blot suggests that there is an increase in the amount of IgG in the CNS from the acute to the chronic phase of infection. To determine if antibodies in the CNS are directed against BDV, CSF and sera from three rats in the chronic phase of infection were analyzed by ELISA for antibodies directed against three BDV-encoded proteins (p40, p23, and gp18) and for capacity to neutralize BDV infectivity. Antibodies were present in the CSF that detected p40, p23 and gp18 and had neutralization activity. However, antibody titers to BDV proteins were lower in the CSF than in the serum (CSF/serum 3-15%). The titers of neutralizing antibodies were also lower in the CSF than in the serum (CSF/serum 16-37%). These results suggest that BDV-specific antibodies are present in the CNS; whether these antibodies play a role in modulation of infection or immunity remains to be determined.

834.3

RETROVIRUS-INDUCED ASTROCYTIC NITRIC OXIDE (NO) PRODUCTION FOLLOWING INFECTION WITH NEUROPATHOGENIC ts1 MoMuLV : A MECHANISM FOR NEURONAL DEATH AND PATHOLOGICAL CHANGES IN SPONGIFORM POLIO-ENCEPHALO-MYELOPATHY OF FVB/N MICE

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Background: NO-mediated neuronal or oligodendroglial damage by HIV-1 in AIDS has been postulated to require NO-producing neurons or macrophages, but the effect of retroviral infection on astrocyte (As) inducible nitric oxide synthase (iNOS) is unknown.

Methods: Subcortical As NO production *in vitro* was monitored by measurement of nitrite formation with Griess reagent before and after ts1 infection. Lipopolysaccharide (LPS) and gamma-interferon (gIFN) effects on mock-infected and ts1-infected As NO production were studied separately and together.

Results: Basal NO production by ts1-infected As was higher than in mock-infected As from 72 hr pi. LPS [10-10,000 ng/ml], but not gIFN, increased NO production in mock-infected and ts1-infected As. LPS [1 ug/ml] / gIFN [100 U/ml] together stimulated significantly higher NO production in ts1-infected As than mock-infected As. L-N-monomethyl-arginine (LNMMA), an inhibitor of iNOS, blocked these effects.

Conclusions: NO production by retrovirus-infected As provides an added source for indirect neuronal/oligodendroglial death.

834.4

***IN VITRO* AND *IN VIVO* ANTIVIRAL ACTIVITIES OF HUMAN RECOMBINANT MONOCLONAL ANTIBODIES TO HSV-1 AND -2**

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Herpes simplex viral disease is an important cause of morbidity and mortality in man. Although the availability of drugs with high therapeutic indexes has greatly improved the management of herpetic infections, the emergence of drug-resistant viral strains, especially in the immunocompromised subjects, has become a cause of serious concern and underscores the importance of developing new and alternative prophylactic and therapeutic tools.

We have isolated large panels of human recombinant monoclonal antibodies to HSV by antigen selection ('panning') from combinatorial phage display libraries. Some such antibodies displayed neutralizing activity *in vitro*. A type common antibody specific for glycoprotein D, antibody ACHSV8, neutralized very effectively both laboratory strains and clinical isolates of HSV-1 and -2 and was capable of inhibiting cell-to-cell transmission of these viruses *in vitro* as an Fab fragment. This antibody acts through first order kinetics and appears to bind monovalently to its epitope. In addition, this antibody more than doubled survival times ($p<0.0001$) when administered to athymic nude mice infected with HSV-1. Such prolongation of survival was seen even when the antibody was administered up to 24 hours post-infection, a time when the virus is already in the PNS.

To improve the implementation of this approach, we have now modified this affinity-based cloning technique to allow for more facile isolation of recombinant antibodies to specific viral proteins in their native conformation. With the modified method, phage display libraries are panned against viral glycoproteins 'captured' from infected cell extracts with immobilized monoclonal antibodies. Using this strategy we isolated six novel neutralizing human recombinant antibodies to HSV glycoproteins D or B. Partially supported by MH - 47680.

834.5

HERPES SIMPLEX VIRUS IN POSTMORTEM MULTIPLE SCLEROSIS BRAIN TISSUE. V.J. Sanders*, A.E. Waddell, S.L. Felisan, W.W. Tourtellotte, M.D., Ph.D. Neurology Research, West L.A. V.A. Med. Ctr., Los Angeles, CA 90073

Objective: To test for the presence of herpes simplex virus 1 and 2 (HSV) in postmortem brain samples from patients with multiple sclerosis (MS) and controls using polymerase chain reaction/Southern blot hybridization methodology (PCR/SB). Background: HSV is a common neurotropic virus capable of long latencies. It can cause focal demyelination in animals. Methods: Dissected plaque tissue classified as active or inactive and unaffected white matter (WM) and gray matter (GM) from 37 MS cases were screened for HSV by PCR/SB. WM and GM from 22 Alzheimer's disease, 17 Parkinson's disease and 22 cases without neuropsychiatric disease served as controls. Results: 46% (17/37) of the MS cases and 28% (17/61) of the control cases had samples positive for HSV ($p=.109$). 41% (9/22) of active MS plaques were positive for HSV. 20% (6/30) of inactive plaques had virus. 24% (9/37) and 14% (5/37) of MS cases had HSV in WM and GM, respectively. 27% (14/61) and 13% (8/61) of non-MS cases had HSV in WM and GM, respectively. No significant differences were found between all subgroups ($p= .097$). Conclusions: HSV was present in a greater frequency of MS cases compared to control cases. A greater frequency of active plaques had HSV present compared to inactive plaques. Virus in MS WM and GM as well as controls makes an association to the MS disease process uncertain. Future: Cellular localization of virus and its relationship to pathology and latency may reveal an association with disease.

834.6

THE USE OF A SMALL ANIMAL MODEL FOR CMV RETINAL INFECTION: IN SITU MOLECULAR BIOLOGY.((E.S. Lazar[1], L Epstein[1], Jr., W. Britt[2], B. Blumberg[1], A. Jones[1], M. del Cerro[1]*))U. of Rochester Medical School[1], Rochester, NY; U. of Alabama, Birmingham School of Medicine[2], Birmingham, AL.

Human cytomegalovirus (HCMV) is an important cause of morbidity and mortality in patients infected with HIV, and little is known about the pathogenesis associated with this virus. No models have been developed to study HCMV infection of human neural tissue in vivo. We have developed an animal model which will aid in delineating this problem. We sought to detect the presence of HCMV in human retinal xenografts in SCID mice using immunocytochemistry (ICC) and In Situ Hybridization(ISH). As HCMV is specific for the human genome, *in vivo* infection of human cells into an animal model is a valuable tool in following the course of the disease. Human fetal retina was transplanted into the anterior chambers (AC) of eyes of SCID mice and infected with HCMV. (ICC) was performed with monoclonal antibodies targeted against early, middle, and late replication phase proteins and other gene products. Peroxidase/anti-peroxidase ICC was performed using an Elite Vector ABC and DAB Peroxidase Substrate Kit. ISH was performed using a 500bp random-primed digoxigenin labeled dsDNA probe which targeted a specific region of CMV DNA. This technique and the process of in situ polymerase chain reaction was tested on AD 169, Coff, and Crump strains of HCMV. 30 days post-infection, the grafts are differentiated with detection of intranuclear and intracytoplasmic viral components. ISH was successful at detecting positive cells with the use of the labelled probe which specifically targeted a region of the CMV genome. This shows that active infection is occurring in the transplanted tissue and that we are infecting human tissue with HCMV. The AD 169 strain was much more reactive than the Coff and Crump strains of HCMV. This animal model allows the study of HCMV replication in nervous tissue and the specific effects of HCMV on human retinal cells. In vitro molecular immunohistochemical and ISH techniques are suited to the detection of HCMV infected retinal cells. Here, we use the AC immune privilege and the immune status of the SCID to mimic HCMV infection in AIDS patients.

Supported by AmFAR and Strong Children's Research Center.

834.7

CELL TYPE-SPECIFICITY AND INDUCIBILITY OF THE HUMAN CYTOMEGALOVIRUS MAJOR IMMEDIATE EARLY PROMOTER IN BRAINS OF TRANSGENIC MICE. J.M. Fritschy*[1], S. Brandner[2], A. Aguzzi[3], B. Lüscher[1], and P. J. Mitchell[2]. [1]Institute of Pharmacology, [2]Institute of Molecular Biology II, University of Zürich, CH-8057 Zürich; [3]Institute of Neuropathology, University Hospital, CH-8091 Zürich, Switzerland.

Human cytomegalovirus (HCMV) has been associated with a variety of pathologies including CNS infections. We showed previously that the HCMV immediate early (IE) promoter directs *lacZ* gene expression in transgenic mouse embryos specifically to sites correlated with known target tissues of congenital HCMV infection in human fetuses (Koedood et al., 1995). Thus, cell type-specific transcription factors that regulate the IE promoter appear to be conserved between humans and mice and to play an important role in determining HCMV infection targets. Here, we analyzed brain-specific IE-*lacZ* expression in our transgenic mice with a view towards identifying potential HCMV infection targets in the neonatal and adult CNS. Immunohistochemical analysis revealed IE-*lacZ* expression in choroid plexus, some endothelial and ependymal cells, and in several neuron populations, notably in brainstem, cerebellum, cerebral cortex, hippocampus, olfactory bulb, and thalamus. Double-labeling with GFAP antibody showed that the IE promoter was inactive in most astrocytes, but was dramatically induced in astrocytes of the ipsilateral cortex and striatum during gliosis induced by a stab lesion of the neocortex. The IE promoter activity pattern in the murine CNS is consistent with clinical data on human HCMV brain infection targets and suggests a model for HCMV progression in the CNS whereby astrocytes become permissive for infection in response to tissue damage caused by infection of primary targets.

834.8

EFFICIENT TRANSDUCTION OF HUMAN NEURONS WITH AN ADENO-ASSOCIATED VIRUS VECTOR. B. Du, P. Wu*, D.M. Boldt-Houle, J. Watters, and E.F. Terwilliger. Divisions of Infectious Disease and Hematology/Oncology, New England Deaconess Hospital, Harvard Medical School, Boston, MA 02215

An adeno-associated virus (AAV) vector containing a lacZ gene under the control of the CMV immediate early promoter was evaluated with respect to its transduction efficiency and integration ability in nondividing human NT neurons. Using dual staining immunocytochemistry with monoclonal antibodies to neurofilament proteins and ß-galactosidase (ß-gal), we were able to demonstrate: 1) the co-localization of neurofilaments and ß-gal in the same cells; and 2) a dosage-dependent pattern of AAV vector transduction efficiency, with up to 100% of the neurons expressing ß-gal. The efficiency found in NT neurons was equal to or greater than the AAV transduction efficiency in 293 cells, which are a highly sensitive cell line to AAV. This indicates that the human NT neurons are readily susceptible to AAV-mediated transduction. No neurotoxicity was detectable by a MTT assay. Further, quantitative PCR analyses of high molecular weight cellular DNA from the transduced neurons indicated that the copy number of the AAV ß-gal genome increased not only in a dose-dependent but in a time-dependent manner, the latter suggesting a slow progressive rate of integration of the vector over a period of days following transduction. In summary, this study demonstrates that AAV-based vectors can efficiently transduce, express and integrate a foreign gene into postmitotic human NT neurons.

834.9

EXPRESSION OF CYTOKINES AND NITRIC OXIDE SYNTHASE BY ASTROCYTES IN CHRONIC DEMYELINATION CAUSED BY MHV-JHM IN SUSCEPTIBLE MICE Ning Sun* and Stanley Perlman Department of Pediatrics and Microbiology, University of Iowa, Iowa City, IA 52242

Intranasal inoculation of C57BL/6 mice with mouse hepatitis virus (MHV), strain JHM under the appropriate condition results in a chronic demyelinating disease characterized clinically by hindlimb paralysis (HLP). Expression of cytokines, nitric oxide synthase (iNOS), MHC class I and II antigen and their possible cellular sources were investigated in this study. Cytokines TNF-α, IL-6, IL-1β and iNOS were all up-regulated in the spinal cords of HLP mice as revealed by immunocytochemistry. Double-label studies demonstrated that cells expressing these cytokines and iNOS were astrocytes. Most astrocytes expressing cytokines and iNOS were not infected by virus but were distributed in the white matter of spinal cord near virus and demyelinating lesions. Also in additional experiments, the spatial relationship of virus-infected cells and the activated astrocytes was analyzed. The analysis showed that 15% of all infected cells in these chronically infected mice was astrocytes. The expression of cytokines and iNOS in astrocytes in these mice is in significant contrast to findings in mice with the acute encephalomyelitis caused by MHV-JHM. In acutely infected animals, TNF-α, IL-6 and iNOS are found mainly in macrophage-like cells. In addition, MHC class I and II antigen are also up-regulated in the spinal cords of chronically infected mice, although not in astrocytes. The results of this study demonstrate that expression of different cytokines and nitric oxide synthase in the astrocytes is a feature unique to the chronic as opposed to acute infection caused by MHV-JHM. The concordance of the present results with findings in other animal models of demyelinating disease and in patients with multiple sclerosis suggests that astrocytes play a major role in chronic demyelination.

834.10

INTRACEREBROVENTRICULAR ADMINISTRATION OF IL-1β IN DOGS PRODUCES CSF LEUKOCYTOSIS BUT NOT FEVER. G.S.F. Ling*, B. Goodman, and D.F. Hanley. Division of Neurosciences Critical Care, Dept. of Neurology, Johns Hopkins Medical Institutions, Baltimore, Maryland 21287.

Il-1β has a well-recognized role in initiating and perpetuating inflammatory cellular responses and in producing fever. Clinically, fever is often the first objective sign of systemic illness. In this study, we examined the effect of intracerebroventricular (i.c.v.) administration of Il-1β on cerebrospinal fluid (CSF) white blood cell count (WBC) and fever.

Beagle dogs (n=6) were each surgically prepared with an indwelling i.c.v. catheter system. After at least one month of recovery, human Il-1β was given i.c.v. in doses of 50, 100 and 200 ng/kg. Controls received 0.1% BSA in normal saline. Blood and CSF specimens were obtained before and for 3 hours after dosing. Body temperature was measured continuously throughout. Biofluid analyses used standard manual methods and an automated hematology counter.

Results demonstrated a rapid increase in CSF leukocyte count. By 5 min. after administration, peak cell counts were obtained. For the 100 and 200 ng/kg groups, the WBCs were 36 ± 8.7 mm^3(Mean $\pm$ SEM) and 89 ± 39.3 mm^3, respectively. These results were significantly different from control ($p < 0.05$, t-test). Over the next hour, these levels gradually decreased back to baseline. At 5 min., the white cells were noted to be predominately mononuclear, approximately 75% of total WBCs. Body temperature did not significantly increase from baseline.

In conclusion, following i.c.v. administration in dogs, Il-1β leads to CSF leukocytosis that is predominantly mononuclear. This effect is dose-dependent. In addition, at the doses tested, there was no pyrogenic response. These findings suggest that disorders that lead to a predominantly CSF monocytosis (such as aseptic meningitis) may be mediated by Il-1β. In addition, it appears that fever requires a higher level of this cytokine or possibly another mechanism is involved.

834.11

NORMAL NEURONAL EXCITABILITY AND SYNAPTIC TRANSMISSION IN PRION PROTEIN GENE ABLATED MICE. P.-M. Lledo*, S. J. DeArmond¶, S.B. Prusiner† and R.A. Nicoll. *Depts. of Cellular & Molecular Pharmacology, ¶Pathology & †Neurology, Biochemistry & Biophysics; UCSF, San Francisco, CA 94143.*

The physiological function of the cellular prion protein (PrP^C) remains obscure. To investigate whether the loss of PrP^C could affect neuronal excitability and/or synaptic transmission in the CNS, we have used hippocampal slices and recorded in the CA1 region from PrP knockout mice ($Prnp^{o/o}$). Field potential recordings have revealed a normal level of synaptic inhibition since in response to stimulation in *stratum radiatum*, responses from $Prnp^{o/o}$ mice consisted of a single population spike similar to the one recorded from control mice and the plot of field EPSP slope versus the population spike amplitude showed no difference between the two sets of data (n = 12). Intracellular recordings also failed to detect any difference in electrical membrane properties and the reversal potential for IPSPs was unchanged (-70.1 ± 1.9 mV in 10 control and -69.5 ± 2.3 mV in 8 $Prnp^{o/o}$ mice). The study of the IPSC kinetics also reveals no modification (n = 14). In another set of experiments, we examined whether LTP was altered and found that no statistical difference existed between the two groups of animals (n = 5). Our findings differ from published results in which the level of inhibition was reported to be impaired in the $Prnp^{o/o}$ mice (Collinge *et al.*, Nature 370: 295-297; 1994), but agree with the earlier detailed behavior studies in which no impairment in $Prnp^{o/o}$ mice could be demonstrated (Büeler *et al.*, Nature 356: 577-582; 1992). *Supported by NATO, the CNRS and the NIMH.*

834.12

THE PRION PROTEIN BINDS TO PSF, AN ESSENTIAL SPLICE FACTOR. Bruno Oesch*, Paul Jenö, and Edith Gubler. Brain Research Institute, University of Zürich, August Forelstr. 1, 8029 Zürich, Switzerland, and Dept. of Biochemistry, Biozentrum, 4056 Basel

Interaction of the prion protein (PrP) with other cellular proteins on ligand blots has led to the identification of two PrP ligands of 45 and 110 kDa (Pli 45 and Pli 110, respectively). Here, we report the identification of Pli 110 as PSF, a splice factor associated with the polypyrimidine-tract binding protein. Pli 110 was purified using sucrose gradient centrifugation followed by chromatography on carboxy methyl cellulose, reverse phase and hydrophilic interaction columns. Tryptic peptides of bovine Pli 110 were sequenced revealing extensive homology to human PSF. Binding to PrP was confirmed with recombinant PSF. Using anti-PSF antibodies, we were able to show the presence of PSF in our most purified preparations of Pli 110. In addition, the binding pattern of PrP to unfixed sections was similar to the distribution of PSF i.e. the signals were strongest in areas of high density of cell bodies.

These results suggest that either PSF may have an alternative function at the membrane or that PrP is not exclusively located at the cell surface. Recently, an intracellular 60 kDa PrP isoform has been identified and shown to be more abundant in scrapie-infected animals suggesting that this isoform may aid the generation of disease-specific PrP^{Sc} or infectious particles. We are currently investigating whether the 60 kDa form of PrP copurifies with PSF.

834.13

NECROTIC, NOT APOPTOTIC, CHANGES IN THE PITUITARY OF FEMALE HAMSTERS INFECTED WITH THE 139H STRAIN OF SCRAPIE. X. Ye*[1], R.I. Carp[2], [1]Div of Neurotoxicology, FDA/NCTR, Jefferson, AR, 72079, [2]NYS Institute for Basic Research for Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY, 10314.

Previous studies have shown that 139H-infection of hamsters causes obesity and marked histopathological changes in the islets of Langerhans and pituitary. Using routine EM, we now report more details of the histopathological changes in the pars distalis of the pituitaries of 139H-infected hamsters. Dilation and vacuolation were first observed in the mitochondria and rough endoplasmic reticulum (RER). The subsequent abnormal events seen included vacuolation and breakdown of the secretory vesicles, lysosomal breakdown and finally cell digestion and lysis. In some damaged cells the nucleus remained attached to the cellular membranes, suggesting that it was protected to some degree from the effects of cytoplasmic toxins by the nuclear membrane. Based upon these observations, we conclude that the cellular death seen in the pituitaries of 139H-infected hamsters is due to necrosis rather than apoptosis.

834.14

THE MOLECULAR PATHOGENESIS OF THE TUBULO-FILAMENTOUS PARTICLES: EVIDENCE OF A HOMOLOGOUS SINGLE-STRANDED DNA IN SPONGIFORM ENCEPHALOPATHY. H. Narang*. Dept. of Psychiatry, School of Medicine, SUNY, Stony Brook NY 11794.

Scrapie, bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease (CJD) with six other diseases have been grouped together as spongiform encephalopathies (SEs). No simple genetic susceptibility factor for SEs has been identified in human or animals. Existence of at least 20 genetically stable strains in SEs appears to be incompatible both with PrP by itself or with a specific point mutation seen in the PrP gene being the agent. The evidence presented so far suggests that SE is an infectious disease and not a genetic disorder. Unique virus-like tubulofilamentous particles termed nemavirus (NVP) have been consistently observed in SE brains by EM. In a blind study, a scrapie-infected and a normal hamster brain were examined at 3, 5, 7, 10, 14, 18, 21, 24 and 28 days post-inoculation. Examination of grids prepared from both left and right sides of the brain at 3, 5 and 7 days revealed no NVP or SAF. However, at 10 days scrapie-infected hamster brains from the inoculated right side revealed both NVP and SAF. From 18 days post-inoculation the NVP and SAF were observed in both sides of the brain which would suggest that replication of the agent starts at the local site of inoculation. No NVP or SAF were seen in any of the preparations from normal brains. Nucleic acid was purified from the content of enriched preparations of mitochondria/NVP. The ssDNA was synthesized into double stranded DNA and inserted in Puc 18, cloned and sequenced. An unusual palindromic six base (TACGTA) 10 repeat sequence was obtained, as observed in scrapie, which revealed no significant homology to other sequences. A probe prepared reacted with a band of about 1.2 kb in scrapie, CJD and BSE but not with control normal DNA specimens.

834.15

BACTERIAL ENDOTOXINS INDUCE NEUROLOGICAL SYMPTOMS IN ADULT WISTAR RATS. R. F. Brown and M. Kiernan.*
Psychology Dept., The University of New South Wales, Australia, 2052.

The present experiments investigated the novel finding that bacterial endotoxins can induce neurological symptoms in adult wistar rats. Neurological symptoms typically appeared 6 - 24 hr post endotoxin administration (ivi. and ip.), persisted for 24 - 36 hr, and then spontaneously resolved. These symptoms appeared in approximately 85 % treated animals, although symptom severity was variable. Mildly affected rats presented with mainly proprioceptive deficits, which manifested as an abnormal gait; moderately affected rats presented with proprioceptive and mild motor deficits; and severely affected rats presented with proprioceptive, motor (paralysis/paresis), and sensory (reflex) deficits. Further experiments were conducted to: investigate the generalisation of this syndrome across a number of different rat strains, and between sexes; localise the site(s) of pathology mediating this syndrome; and, determine which endotoxin-induced chemical pathway(s) might be mediating this syndrome.

834.16

PNEUMOCOCCAL CELL WALL COMPONENTS RELEASE TNF-α FROM CEREBRAL ASTROGLIAL AND ENDOTHELIAL CELL CULTURES M. Weih*, D. Freyer, J. R.Weber, P. Scholz, A. Meisel, K. Angstwurm,W. Bürger, U. Dirnagl. Dept. of Neurology, Dept. of Microbiology, Charité, Humboldt University Berlin and Schering AG, Berlin, Germany.

Astroglial cells and cerebral endothelial cells have been shown to produce tumor necrosis factor-alpha (TNF-α) upon stimulation with cytokines like IFN-γ, IL1-β or bacterial lipopolysaccarides (LPS). The cell wall of Streptococcus pneumoniae (PCW) has been shown to cause meningeal inflammation,which might be mediated in part by pro-inflammatory cytokines like TNF-α. TNF-α has also been found in cerebrospinal fluid in experimental menigitis and in humans. We tested whether astroglial or endothelial cells could account for this TNF-α release. Addition of PCW to cultured rat astroglial cells increased TNF-α in the supernatant significantly after 4h from 3 ± 0,63 pg/ml to 9,8 ± 1,6 pg/ml and was inhibited by dexamethasone (10^{-6} M). TNF-α release from astroglial cells, as determined by the L929 bioassay, was dose-dependent in a range shown to cause meningeal inflammation in vivo. TNF-α production in cerebral endothelial cells after 12 h was higher than in astrocytes (117 vs. 45 pg/ml) and also inhibitable by dexamethasone.Our results suggest astroglial, microglial and endothelial cells as sources of TNF-α, a pro-inflammatory cytokine, which could mediate vascular and antimicrobial response in the early phase of experimental pneumococcal meningitis leading to recruitment of blood leucocytes and finally breakdown of the blood-brain-barrier (This work was supported by the DFG).

834.17

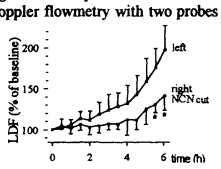
IS THERE A ROLE OF NEUROGENIC INFLAMMATION IN THE rCBF RESPONSE IN BACTERIAL MENINGITIS? J. R. Weber, K. Angstwurm, R. Empson*+, G.M. Bove #, K.M. Einhäupl, U. Dirnagl and M.A. Moskowitz #.
Depts. of Neurology and +Physiology, Charité, Humboldt University, 10098 Berlin, Germany, #Massachusetts Gen. Hosp., Harvard Medical School, Boston.

Headache and nausea are the most relevant clinical symptoms in early bacterial meningitis (BM). The hypothesis that headache in BM may result from trigeminal activation is strongly supported by observations in the early phase of experimental meningitis which is characterized by a dramatic regional cerebral blood flow (rCBF) increase and inflammation within the first 6 hours. We undertook this study to examine a possible role of trigeminal axons in the rCBF response.

In 5 male wistar rats the nasociliary nerve (NCN), which innervates the meninges over the frontal lobe was cut on the right side 10 days before the experiment. Meningitis was induced by intracisternal injection of pneumococcal cell wall components (equivalent of 10^7 cfu Streptococcus pneumonae PnR-527, Jena). Physiological parameters were monitored throughout the experiment. rCBF was measured continuously over 6 hours with laser Doppler flowmetry with two probes bilaterally over the right and the left frontal lobe of the cortex through the thinned skull

LDF % / side	*0h*	*3h*	*6h*
right, NCN cut	100	123 ± 7	197 ± 29
left	100	106 ± 19	140 ± 17*

(p< .05) Students t-test, mean ± SD

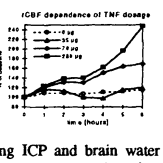


Denervation of the meninges attenuates the rCBF increase in BM. The study provides first evidence for a role of a neurogenic mechanism in rCBF response in acute inflammation of the CNS. We speculate that neuropeptides released from trigeminal C-fibers may be involved in mediating rCBF increase in BM.

834.18

INTRACISTERNAL INJECTION OF TUMOR NECROSIS FACTOR ALPHA (TNFα) INCREASES REGIONAL CEREBRAL BLOOD FLOW (rCBF). K.Angstwurm, M.Weih, D.Freyer, K.M. Einhäupl, P.Scholz[1], G.Ransmayr[2]*, U.Dirnagl, J.R.Weber. Dept. of Neurology, Humboldt-University, [1]Schering AG, Berlin, Germany,[2]Dept. of Neurology, Univ. of Innsbruck, Austria.

TNFα is a pleiotrophic cytokine, which is thought to be a central mediator of various CNS diseases. Its role in bacterial meningitis, for example, is still under controversial discussion. We tested the effect of intracisternal injection of recombinant rat specific TNFα on rCBF, intracranial pressure (ICP), pleocytosis and brain water content in rats. TNFα (activity: 3 10^7 units/mg) was applied in three dosages (35µg (n=3); 70µg (n=4); 280µg (n=3) per animal). In control animals saline i.c. was injected. rCBF measurement was performed by laser Doppler flowmetry through the thinned right parietal bone. Within the observed 6h post i.c. injection no differences were seen in blood pCO2, pO2, and pH of treated and untreated rats. In TNFα i.c. injected animals a systemic effect was leukopenia. Otherwise the 35µg TNFα group did not differ from saline i.c. injected. An injection of 70µg or 280µg TNFα increased rCBF significantly beginning 2h after i.c. injection compared to the lower dosages. 4,5h and later rCBF of 280µg TNFα group was higher than of all other groups (mean±SD 6h after i.c. injection: 0µg: 115±17; 35µg: 120±10; 70µg: 170±39; 280µg: 248±28. p<0,05, ANOVA, Student-Newman-Keuls). Animals with the two higher dosages showed mild pleocytosis (400/µl, p<0,05). No significant differences were found concerning ICP and brain water content. Conclusions: 1.CNS inflammation requires more mediators than just TNFα, 2. TNFα is a possible mediator for systemic effects of CNS inflammation. The role of NO in TNFα mediated rCBF response has to be eluciated in future.



834.19

TROPHISM OF *TOXOPLASMA* FOR ASTROCYTES IN A HUMAN FETAL NEURAL CULTURE. S.K. Halonen and F.C. Chiu*. Dept. of Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10128.

Toxoplasma gondii is an intracellular parasite that is one of the most common opportunistic infections of the CNS in AIDS patients, causing an often fatal encephalitis. Treatment of this disease is hampered by a lack of information about the basic biology of this parasite in neuronal cells. In this study primary cultures from human fetal brain were used to study the behavior of Toxoplasma in neural cells. Toxoplasma was able to infect both astrocytes and neurons but growth of the parasite in astrocytes was approximately 10-fold higher than in neurons. The behavior of Toxoplasma also differed drastically in astrocytes vs. neurons. In astrocytes, Toxoplasma replicated every 4-6 hrs, with an average of 22-24 parasites/host cell at 48 hrs. In neurons however, parasites replicated only 1-2 times over this same 48 hr period. There was evidence that some of the infected neurons were necrotic 24 hrs after infection. However, approximately 10% of the infected neurons were able to support replication of the parasite. These results indicate that 1) astrocytes support the majority of the proliferation of the parasite in the brain and 2) neurons exhibit a differential susceptibility to infection with Toxoplasma. A better understanding of the trophism for astrocytes and of the differential behavior of Toxoplasma in neurons may yield insights into the mechanisms underlying toxoplasmic encephalitis.
(S.K. Halonen is a Aaron Diamond Foundation Fellow and this work was supported by a grant from The Aaron Diamond Foundation).

MENTAL ILLNESS—SCHIZOPHRENIA III

835.1

NMDA RECEPTOR DOMAINS IN POSTMORTEM BRAINS OF SCHIZOPHRENIC PATIENTS. T. Matsunaga*[1], A. Shimada[2], A. G. Mukhin[1], D. K. Ingram[2], S. R. Zukin[3], J. Kleinman[4], M. Casanova[5] and E. D. London[1]. Intramural Research Programs, [1]NIDA and [2]NIA, Baltimore, MD 21224; [3]Albert Einstein Col. Med., New York, NY; 10461; [4]Intramural Research Program, NIMH, Washington, DC 20032; and [5]Med. College of Georgia, Augusta, GA 30912.

Growing evidence suggests that aberrant glutamatergic systems interacting with dopaminergic systems may be involved in the pathophysiology of schizophrenia. The NMDA receptor is a heteropolymeric complex that has distinct domains, including NMDA and glycine recognition sites and channel binding sites. Furthermore, the subunit composition of the receptor displays regional variations in brain.

[H-3]CGP39653, [H-3]dichlorokynurenic acid and [H-3]dizocilpine were used to assay specific binding to NMDA and glycine recognition sites and channel sites, respectively, in membranes from the orbital gyrus (OG) and superior temporal gyrus of postmortem brain samples from normal control subjects and patients with schizophrenia and bipolar disease.

The specific binding of [H-3]CGP39653 to OG membranes was higher in samples from schizophrenic and bipolar patients (+66%) than that in controls. The difference between the schizophrenic and control samples was significant ($p<0.05$ by ANOVA and Scheffe's test). There were no other statistically significant differences. Correlations between specific binding of any of the radioligands in either brain region and the intervals between death time and freezing time were not statistically significant.

These findings are consistent with frontal cortical glutamatergic hypofunction and consequent up-regulation of NMDA recognition sites in schizophrenia, but do not exclude the possibility that the effect seen is due to neuroleptic treatment.

835.2

DISTRIBUTION OF CCK mRNA IN PREFRONTAL CORTEX OF SCHIZOPHRENICS. T.Takahashi[1,2], H.Arai[2], R.Inoue[2], A.Matsumoto*[2],†, P.Mckenna[3] and P.C.Emson[1] 1)Dept. of Neurobiol., The Babraham Inst., Babraham, Cambridge CB2 4AT, UK 2)Dept. of Psychiat. & Anat. †, Juntendo Univ. Sch. of Med. Hongo, Tokyo 113, Japan 3)Dept. of Psychiat., Univ. of Cambridge, Cambridge CB2 2QQ, UK

Cholecystokinin(CCK) mRNA is present in large amounts in the human cerebral cortex. CCK-immunoreactivity and mRNA has also been detected in dopaminergic neurons of the substantia nigra, pars compacta and the ventral tegmental area suggesting a possible involvement of CCK in the pathology of schizophrenia. In this study we have used *in situ* hybridization technique with an antisense oligonucleotide probe specific for CCK mRNA to detect sites of CCK mRNA in post-mortem human prefrontal cortex (Brodmann areas 9, 10 and 11). The distribution and numbers of cells expressing CCK mRNA have been determined using computer assisted image analysis in brains from neurologically normal controls. CCK mRNA was expressed with similar laminar pattern in all cases. On film autoradiography there was no significant differences in amounts of CCK mRNA between schizophrenics and controls, however that of schizophrenics tended to be reduced than controls. On emulsion autoradiography CCK mRNA was expressed in large amounts in populations of small neurons in cortical layers II-VI, in addition a number of larger pyramidal cells in layers II and V also contained CCK mRNA albeit at lower levels. As the results of counting silver grains, schizophrenics were significant reduced in cellular levels of CCK mRNA in layer V and VI in area 9, in layer III in area 10, and in layer III in area 11. These results suggest that the ability of CCK biosynthesis in the region of prefrontal cortex of schizophrenics may be downregulated.

835.3

WITHIN-SUBJECT CHANGES IN STRIATAL D2 RECEPTOR BINDING POTENTIAL ARE RELATED TO CHANGES IN SCHIZOPHRENIC SYMPTOMS. M.B. Knable*, R. Coppola, D.W. Jones, J. Gorey, K.S. Lee, D.R. Weinberger. NIMH, IRP, CBDB, 2700 M.L. King Jr. Ave., S.E., Washington, D.C., 20032

Ten schizophrenic patients underwent prolonged IBZM SPECT studies while free from neuroleptic drugs (mean 150; range: 7- 730 days) and a measure proportional to peak D2 receptor binding potential (BP) was determined as previously described (Knable, et al., J. Nuc. Med., 1995). Sixteen healthy controls were studied for comparison. For schizophrenic patients, the peak BP for the left and right striata were 0.67±0.25 and 0.68 ±0.20 respectively, and for normal controls these values were 0.73±0.21 and 0.71±0.21. Although peak BP tended to be less in schizophrenics, this difference was not statistically significant. There were no significant differences between the left and right striata for either group. There were no significant correlations between peak BP and positive symptoms, negative symptoms, movement disorder, age, or illness duration. Four of the schizophrenic patients were able to complete two SPECT studies during the drug free period. Percent change in peak BP was significantly correlated with change in positive symptoms (rho=0.80) and change in negative symptoms (rho=1.0). This association was not explained by persistent D2 receptor occupancy by neuroleptic drugs during the first scan, since there was an inverse relationship between the drug free period at the first scan and change in peak BP. These data suggest that increased D2 receptor density is not present as a trait marker in schizophrenia, but that D2 receptor number covaries with the severity of certain symptoms characteristic of the illness.

835.4

PERINATAL IBOTENATE INJECTIONS PRODUCE ANATOMICAL AND BEHAVIORAL CHANGES SIMILAR TO SCHIZOPHRENIA. R. A. Devo*, J. L. Gerson, K. L. Marshall, and J. M. Hittner. Dept. of Psychology, Winona State Univ., Winona, MN 55987.

The glutamate theory of schizophrenia proposes a relationship between degeneration of glutamatergic neurons and the behavioral deficits associated with schizophrenia. This study evaluates the effects of perinatal lesions of hippocampal glutamatergic neurons and subsequent changes in learning and social behavior.

Twenty-two male Long-Evans hooded rats received bilateral hippocampal injections of ibotenic acid (IBO) or PBS (controls) on day five postpartum.

Social-Play Testing (Day 28). Rats were individually housed for 72 hr prior to testing. IBO rats played significantly less (made fewer pins) than controls $F(1,19)= 4.70$, $p<.05$.

Eight arm radial maze training (Day 70). Subjects were placed in the hub and allowed to enter each arm for 10 sessions. Performance was significantly poorer in the IBO group $F(1,18) = 16.927$, $p<.001$.

Extinction testing (Day 80). Rats were trained to barpress to a criterion of 400 responses followed by 3 extinction sessions. IBO treated rats demonstrated resistance to extinction.

Histology. Brains were fixed (10% formalin), sectioned (20 μm) and stained using cresyl violet. IBO decreased the size of the dorsal hippocampus and enlarged the lateral ventricles.

These data suggest that hippocampal IBO injections produce anatomical and behavioral deficits similar to schizophrenia.

835.5

M1, M2, M3 DISTRIBUTION IN THE THALAMUS OF POSTMORTEM HUMAN BRAIN

J. H. Callicott, A.M. Murray, T.M. Hyde, A.de Bartolomeis, M.M. Herman, R.C. Saunders,* J.E. Kleinman
CBDB, NIMH Neuroscience Center at St. Elizabeths, NIH, Washington, D.C., 20032

The thalamus is a crucial synaptic relay point for limbic system processing. In particular, the anterior and medial thalamic nuclear groups are key intermediaries in the connections between medial temporal and frontal cortical areas. As part of an ongoing investigation of neurochemistry of schizophrenia and suicide, we studied the distribution of muscarinic receptor subtypes in the "limbic" subdivisions of the thalamus -- anterior and mediodorsal nuclear groups.

Using quantitative autoradiography, we labeled M1 ([^{3}H]pirenzepine), M2 ([^{3}H]NMS), and M3 ([^{3}H]NMS) receptors in human postmortem tissue for normal controls, schizophrenics, suicides, and neuroleptic controls. Using one factor ANOVA comparisons, M2 and M3 receptors were of equal abundance and both greater than M1 in anterior and mediodorsal thalamic nuclei in the normal control group. Similar patterns were observed in the schizophrenic and neuroleptic control groups. The only significant finding was a marked elevation of M1 receptors in both the anterior and mediodorsal thalamic nuclei of the suicide group when compared to the other groups. M1 receptor density in suicides was elevated approximately 4-fold over controls. Urine toxicology screens for all of the suicides were negative for tricyclics or neuroleptics, thus suggesting that this relative upregulation of M1 receptors may be a reflection of underlying affective illness rather than medication effect. These results conform to the pattern of M1receptor changes in suicide in human striatum previously reported by Murray et al. (submitted).

835.6

GABA-A RECEPTOR BINDING CHANGES IN SCHIZOPHRENIA: DORSOLATERAL PREFRONTAL AND CINGULATE CORTICES

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GABA neurons constitute the major subtype of intraneuron in the human cortex. Reports of GABA-A receptor abnormalities in schizophrenia have major implications since GABA intraneurons mediate 5HT input and glutamatergic output in the cortex, both of which are reportedly abnormal in schizophrenia. Furthermore, there have been reports of abnormalities in cortical GABA-A receptors density in schizophrenia. Tissue mounted 14 µm thick sections from DSMIII-R diagnosed schizophrenics (n=8, mean age=59.25±5.3, PMI=19.4±3.8), normals (n=9, mean age=54.8±5.2, PMI=25.5±4.5), non-schizophrenic suicides (n=9 mean age=57.2±7.9, PMI=20±10), and neuroleptic treated controls (n=7, mean age=51.8±7.2, PMI=30.0±6.9), were used for receptor autoradiography of dorsolateral prefrontal cortex and anterior cingulate cortex. Binding was carried out according to the method of Benes et al., (1992) with 5 nM [^{3}H]muscimol as the radioligand. Non-specific binding was determined in the presence of 10 µM GABA or 100 µM (+)-bicuculline. For purposes of anatomical analysis the pre-frontal cortex was divided into layers II-III and IV-VI. The cingulate cortex was analyzed in three divisions (layers I, II-III and IV-VI). There was no difference between schizophrenics and control groups for GABA-A receptors in dorsolateral prefrontal or anterior cingulate cortex. Scatchard analysis was carried out on a separate cohort of schizophrenics (n=12, mean age=54.5±4.6, PMI=17.4±8.0), and controls (n=9, mean age=47±4.4, PMI=18±1.8), in anterior cingulate cortex. Homogenate binding was performed using the ultracentrifugation method of Yang and Olsen, (1987). Again [^{3}H]muscimol (1.25-35nM) was used as the radioligand. Non-specific binding was determined by 100 µM GABA. There was no difference in kD and Bmax between controls and schizophrenics. Non-specific binding was considerably higher when (+)-bicuculline was utilized compared to GABA, using both autoradiography and homogenate binding techniques. This data does not support the notion that GABA-A receptor density is abnormal in dorsolateral prefrontal or cingulate cortex in schizophrenia.

835.7

CHOLINERGIC NEUROCHEMISTRY OF THE CEREBRAL CORTEX IN SCHIZOPHRENIA.

S.M. Gabriel*, V. Haroutunian, P. Powchik, M. Davidson, K. L. Davis. Departments of Psychiatry, Mount Sinai School of Medicine, New York, NY 10029 and Bronx VA Hospital, Bronx, NY 10468.

A common symptom of chronically institutionalized elderly schizophrenics is severe cognitive impairment. To determine the neurochemical correlates of cognitive dysfunction in elderly schizophrenics, neurochemical studies were performed in 41 schizophrenic patients receiving extensive cognitive and neuropsychiatric evaluations in life. Cases were specifically selected to be free of neuropathologic lesions and to vary extensively with respect to the cognitive dementia rating (CDR) scale and to the positive and negative syndrome scale (PANSS). Of the ten cerebral cortical regions examined, we found that cognitive decline in elderly schizophrenics was correlated with the activity of the acetylcholine marker enzyme, choline acetyl transferase (ChAT), in the parietal cortex only. Levels of ChAT in schizophrenia were not comparable to the reduced levels observed in Alzheimer's disease. These data indicate that diminished cholinergic activity may contribute to the severe cognitive impairment associated with schizophrenia.

835.8

GENERATING MICE OVEREXPRESSING D4 DOPAMINE RECEPTOR AND LACKING NMDA RECEPTOR NR2B SUBUNIT BY TRANSGENIC TECHNIQUE.

Toshikuni Sasaoka*, and Susumu Tonegawa, Center for Cancer Research, MIT, Cambridge, MA 02139

According to a current dopamine hypothesis of schizophrenia the hyperactivity in the mesolimbic dopamine system contributes to schizophrenic symptoms. To study the involvement of hyperactivity in mesolimbic dopamine system in schizophrenia we have used a transgenic mouse approach to generate mice overexpressing the D4 dopamine receptor (DRD4). Growing evidence suggests the contribution of an abnormal glutamate system to schizophrenia. Phencyclidine, which results in effects that resemble schizophrenic symptoms, is a non-competitive antagonist of the NMDA receptor. As the NMDA receptor subunit NR2B is expressed specifically in the forebrain, disruption of the NR2B gene could ablate NMDA receptor function selectively in the forebrain and thus affect glutamate-mediated synaptic transmission in the region. We have used the gene targeting approach to mutate the NR2B gene in mice.

We made the human DRD4 transgene construct containing 12.5 kb of 5'-flanking region, 4 kb of the whole coding sequence and 1 kb of 3'-flanking region. The transgene construct was introduced into (C57BL/6J x CBA/J) F2 eggs by microinjection. We generated 8 DRD4 transgenic lines. The expression levels of the DRD4 transgene are being assessed.

D3 ES cells were transfected with the NR2B targeting construct in which the coding sequences of transmembrane domain 4 were deleted by replacement with a neomycin resistance gene. After selection in G418, one targeted clone out of 192 G418-resistant clones was identified by Southern analysis. Chimeric mice were generated with this ES clone, and bred to get ES cell derived progeny. Preliminary data on the phenotype of the mutant mice will be presented. (Supported by Stanley Foundation)

835.9

ANALYSIS OF DOPAMINE (D2, D3 AND D4) RECEPTORS AND DOPAMINE TORANSPORTER GENE VARIANTS IN SCHIZOPHRENIA.

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We report data from four studies on variants of the D2 receptor gene (Ser311→Cys), BalI polymorphism in D3, 48-nucleotide repeat Polymorphism in D4, and 40-nucleotide repeat polymorphism in the dopamine transporter gene, in patients with schizophrenia (Sc) (n=69), mood disorder (n=10), neurological disease (n=40) and controls (n=29). Each polymorphism was typed by amplification of genomic DNA by PCR and agarose gel electrophoresis. Seven individuals were heterozygous for Cys/Ser311 of D2 and two of them were Sc patients with a family history of the illness. Two patients with Sc were homozygous for the mutant form of D3 and showed a stuporous state at disease onset. We detected four-fold and two-fold repeats in D4, and ten-fold, nine-fold and seven-fold repeats in the dopamine transporter gene. There was no evidence for association between each variant and reactivity to pharmacotherapy for Sc.

835.10

SUBICULAR MAP-2 IMMUNOREACTIVITY IN SCHIZOPHRENIA.

G. Rosoklija, M.A. Kaufman, D. Liu, A.P. Hays, N. Latov, C. Waniek, J.G. Keilp, A. Wu, S.A. Sadiq, J. Gorman, I. Prohovnik, A.J. Dwork*, N.Y. State Psychiatric Institute and Columbia University, New York, NY 10032

We studied MAP-2 immunoreactivity on paraffin sections of hippocampal formation from autopsies of 15 unselected schizophrenics, mean age 73, 14 unselected psychiatric controls, mean age 78, and 6 non-psychiatric patients without neuropathological abnormalities, mean age 51. Psychiatric diagnoses were determined by standardized review of clinical records (Keilp *et al.*, Schiz. Res., in press). Complete neuropathologic examinations were performed and included thioflavine S stain and immunohistochemistry with Alz 50 and an antibody to paired helical filaments. Immunoperoxidase staining for MAP-2 was performed on paraffin sections, and intensity was evaluated by densitometry. Among the schizophrenics, immunoreactivity in subiculum was significantly lower than in CA4 ($p<.05$, 2-tailed paired t-test), while in each control group, immunoreactivity was slightly more intense in subiculum than in CA4 (not significant). The difference between immunoreactivity in subiculum and immunoreactivity in CA4 was affected by a diagnosis of schizophrenia but not by age, neuropathologic diagnosis, post mortem interval, or senile plaque or neurofibrillary tangle counts. Pronounced loss of subicular immunoreactivity was present in 6 (40%) of the schizophrenics, 3 (21%) of the psychiatric controls, and none of the non-psychiatric controls. Thus, in our hands, loss of subicular MAP-2 is strongly associated with schizophrenia but is not a specific or sensitive marker. Our results extend the original observation of this association by Arnold *et al.* (PNAS 88:10850, 1991). The association of schizophrenia with diminished subicular MAP2 immunoreactivity is one of the few results in the post mortem study of schizophrenia to be confirmed in independent laboratories studying different samples of subjects. *Support: AG 10638 & The Stanley Foundation.*

835.11

DETECTION OF SENILE DEGENERATION IN OLD BRAIN SPECIMENS FROM A PSYCHIATRIC COLLECTION. A.J. Dwork, D. Liu*, M.A. Kaufman, I. Prohovnik. N.Y.S. Psych. Inst. & Columbia U, New York, NY 10032

Conflicting reports have been published regarding the prevalence of neuropathologic changes of Alzheimer's disease (AD) in brains of older individuals who suffered from schizophrenia. Large samples and valid histologic techniques are needed to resolve this issue; for example, an observed AD rate of 5% among controls and 10% among schizophrenics would require over 200 subjects in each group to be statistically significant ($X^2 = 3.841$, df = 1, $p < .05$). Psychiatric brain samples of this size can in general be obtained only from collections of specimens accrued over many years. Since we are in the process of studying AD in such a collection, we began by determining which staining procedures could be applied to material that had been in formalin or paraffin for many years. We found that: (1) Plaque and tangle counts from contemporary thioflavine S staining on tissue that had been in formalin for 50 years or paraffin for at least 31 years (the oldest samples examined) were entirely consistent with those obtained from von Braunmuhl stains performed at the time of the original neuropathologic examinations. (2) In the same specimens, senile degenerative changes were easily recognized by immunohistochemistry with antibodies to paired helical filaments, ß-amyloid, or ubiquitin. (3) Compared to thioflavine S staining, immunoreactivity with Alz 50 was well demonstrated after 9 years in formalin, weaker after 20 years in formalin, and absent after 30 years in formalin. Alz 50 immunoreactivity was well preserved in paraffin blocks 31 years old. (4) In contrast to staining for senile degenerative changes, immunohistochemistry for MAP2 and synaptophysin was significantly impaired after several years of storage in formalin. We conclude that a variety of staining techniques for senile degenerative changes can be applied reliably to archival material, but that each method must be validated individually. *Supported by AG 10638.*

835.12

CHOLINERGIC PEDUNCULOPONTINE NEURONS IN SCHIZOPHRENIA: FAILURE TO FIND INCREASED CELL NUMBERS. K.F. Manaye*, R. Zweig, D. Wu and D.C. German. Dept Psychiat, UT Southwestern Med Sch, Dallas, TX 75235-9070, Dept Biochem, Univ Kentucky, Lexington, KY 40536, and Dept Neurol, LSU Med Cntr, Shreveport, LA, 71130.

The number of cholinergic pedunculopontine (PPN) neurons has been reported to be increased in the brains of schizophrenics, based upon examination of post-mortem brains with a stain for NADPH-diaphorase (Karson et al., 1991). The present study sought to examine PPN neurons using an antibody against choline acetyltransferase (ChAT), and computer imaging procedures to map the cell locations. In 3 schizophrenic and 6 control cases, there were no differences in the total number of ChAT-containing cells in the Ch5d, Ch5c or Ch6 regions; in controls there were about 20,000 total cells, unilaterally. Also, in 8 schizophrenic and 11 control cases, there was no difference in the number of Nissl-containing PPN cells through the dense portion of the nucleus (Ch5c); in schizophrenic cases there were 577 ± 97 (mean ± SEM) cells per section, and in control cases there were 583 ± 70. In both control and schizophrenic cases, there were twice as many Nissl-stained cells as ChAT-immunostained cells. These data do not support the hypothesis that there are increased numbers of cholinergic PPN neurons in schizophrenia.

835.13

DIFFERENTIAL REGULATION OF $GABA_A$ AND BENZODIAZEPINE RECEPTOR BINDING IN THE HIPPOCAMPAL FORAMTION OF SCHIZOPHRENICS. S.L. Vincent*, Y. Khan, R. Wickramasinghe, and F.M. Benes. Department of Psychiatry and Program in Neuroscience, Harvard Medical School and Laboratory for Structural Neuroscience, McLean Hospital, Belmont, MA 02178.

Recent postmortem studies have reported a marked upregulation of $GABA_A$ receptor binding (RB) activity in the anterior cingulate and prefrontal cortices of schizophrenic (SZ) subjects. Because the hippocampal formation has also been implicated in the pathophysiology of SZ, the current study has examined $GABA_A$- and benzodiazepine- (BZ) RB in this region from control (N=15) and SZ (N=8) subjects. Low-resolution analysis of $GABA_A$-RB showed an increase for SZs in the area dentata, CA4, CA3, CA2, subiculum, and presubiculum. The magnitude of these differences ranged from 22% to 91%, and was largest for stratum oriens (91%) and stratum pyramidale (57%) of CA3, and lowest in all layers of CA1 (22-36%). In contrast, BZ-RB showed more modest increases in CA3 (stratum oriens, 32%), CA1 (stratum oriens, 13%), subiculum (25%), and presubiculum (48%). High-resolution analysis of $GABA_A$-RB for CA3 showed no change on pyramidal cells (PNs), but a 170% increase on nonpyramidal cells (NPNs), while for CA1 there was a 43% increase on PNs and no change on NPNs. In contrast, a similar analysis of BZ-RB showed no change on either PNs or NPNs of CA3. These differential changes of $GABA_A$-RB by subregion and cell type are consistent with a model in which there are discreet alterations in disinhibitory GABAergic modulation in CA3 of SZs. Although $GABA_A$ and BZ binding sites are believed to share the same macromolecular receptor complex, the present finding of a disparity for RB-activity between these two sites suggests they may be differentially regulated in SZ brain. Supported by MH00423, MH42261, MH31154, and the Scottish Rite Foundation.

835.14

Gene expression for NMDA receptor subunits in the prefrontal and parieto-temporal cortex of schizophrenics and controls. E.G. Jones[1] S.Akbarian[1]* D. Bradley[1], D.Trinh[1], S.G. Potkin[2], W.E. Bunney Jr[2], N.J.Sucher[3]. *[1]Dept. of Anatomy and Neurobiology and [2]Dept. of Psychiatry and Human Behavior, Univ. of California, Irvine, CA 92717 and [3]Dept. of Neurology, Children's Hospital, Harvard Medical School, Boston MA 02115*

NMDA receptor channel blockers potentially induce a psychosis with psychopathological symptomes that are frequently observed in patients with schizophrenia, but evidence for a defect in NMDA receptor mediated signal transmission in brains of schizophrenics has remained inconclusive. Using in situ hybridization histochemistry and optical densitometry, the present study analysed in 15 schizophrenics and 15 matched controls the genetic expression pattern of all 5 NMDA receptor subunit genes (NR1, NR2A-D) in the prefrontal cortex, a brain region thought to be primarily involved in the pathophysiology of the disease. Comparative studies were conducted on the parieto-temporal cortex of the same specimens. Both in schizophrenics and controls, levels of mRNA for NR1, NR2A, NR2B and NR2D subunit polypeptides were overall 40% higher in the prefrontal cortex, in comparison to the parieto-temporal cortex. The prefrontal , but not the parieto-temporal cortex of schizophrenics showed a signficant change in the stoichiometric composition of mRNAs for the NR2 subunit gene family, due to a selective increase in the proportion of NR2D subunit mRNA (schizophrenics: 34.6 ± 4.0%, controls: 23.4 ± 2.5%, $p = 0.031$). Given the fact that NR2D subunit appears to enhance the excitability of the NMDA receptor (Monyer et al., 1994), the increased expression of the mRNA for NR2D polypeptide in the prefrontal cortex of schizophrenics may be a compensatory response to the functional hypoactivity and to the assumed deficits in NMDA receptor mediated signal transmission. Supported by grant no. MH 44188 from the NIMH.

835.15

CHANGES IN GENE EXPRESSION IN RAT BRAIN AFTER CHRONICAL NEUROLEPTIC TREATMENT. N. Dahmen, V. Fischer, M. Fickova, S. Reuss, G.D. Bartoszyk* and C. Hiemke. Departments of Psychiatry and Anatomy, University of Mainz, Germany and Department of CNS-Research*, E. Merck, D-64271 Darmstadt, Germany

The administration of antipsychotic drugs has been demonstrated in rat and human brain to alter the pattern of gene expression. Among the genes regulated are those that encode for dopamine receptors, various neuropeptides and immediate early genes. To test the hypothesis that further changes in neuronal gene expression are involved in the longterm effects of chronically administered neuroleptic drugs, rats were treated orally for 3 weeks with haloperidol, 2 mg/kg per day, and clozapine, 100 mg/kg per day. After that time, animals were decapitated and haloperidol and clozapine serum levels measured in truncal blood by radio receptor assay and HPLC, respectively. Total RNA was extracted from whole brains and subjected to RNA differential display. This method is a tool to identify and clone differentially expressed genes and involves the reverse transcription of mRNAs with oligo-dT primers anchored to the beginning of the poly(A) tail, followed by PCR reaction in the presence of a second 10mer arbitrary in sequence and analysis of amplification products on a sequencing gel. Comparison of gel patterns derived from treated and control animals resulted in reproducible differences in the three groups. In particular, one transcript was observed that only appeared in samples derived from control and haloperidol treated animals but not from clozapine treated animals. Our results support the study hypothesis of a modulation of neuronal gene expression by neuroleptic drugs and suggest that gene regulation is relevant to the treatment and/or the pathophysiology of schizophrenia.

835.16

DEVELOPMENTAL EXPRESSION OF ALPHA-3 INTEGRIN, A MOLECULE IMPLICATED IN SCHIZOPHRENIA. **Vicente A. Honer WG, Nobrega J*, Kennedy JL. Clarke Institute of Psychiatry, Toronto, Ontario M5T 1R8, Canada.**

Accumulating structural and molecular evidence suggests that a neurodevelopmental disturbance may be a cause of schizophrenia. In brains of schizophrenics, several limbic structures show altered cell numbers and/or disrupted cell orientation. Also, several neurodevelopmental molecules are changed in these regions, such as NCAM, NMDA receptors, and synaptic vesicle proteins. We have been investigating the role, in schizophrenia, of molecules involved in brain development and in the synaptic plasticity events that occur during brain maturation. Using an antibody that binds differentially to schizophrenia versus control brain homogenates, a human cDNA clone, WH4, was isolated and showed a 96% homology to the α_3 subunit of $\alpha_3\beta_1$ integrin. Integrins are cell membrane receptors that bind the extracellular matrix, promoting communication between the intra and extracellular environments, and are crucial for cell movement. We have found strong α_3 subunit (WH4) expression in rat brain and have analysed its mRNA distribution in the adult animal, and during postnatal development by *in situ* hybridization. We have found a widespread distribution of the mRNA throughout the adult brain, with higher levels in hippocampus, pyriform cortex, amygdala, hypothalamus and cerebellum. Preliminary results indicate higher levels of expression in 1 day old rat brains, which decrease to adulthood levels after 7 days. This pattern of expression is consistent with a role of the molecule in plasticity events during brain maturation and the involvement of α_3 integrin in schizophrenia remains intriguing .

835.17

LOCALIZATION OF D4 DOPAMINE RECEPTORS IN RAT BRAIN. A.M. Murray*, T.M. Hyde, B.K. Lipska, G.K. Wood, J.E. Kleinman and D.R. Weinberger, Clinical Brain Disorders Branch, IRP, NIMH, St. Elizabeths Hospital, Washington, DC 20032.

Studies in human brain have demonstrated a mismatch for mRNA localization for D4 receptors. There is neglible D4 mRNA in the striatum/nucleus accumbens but abundent D4 receptor binding. It is possible that D4 receptors are not synthesized in the basal ganglia, but rather have a presynaptic localization on afferent terminals in the striatum. Prefrontal cortex contains the largest amount of D4 mRNA in the human brain. If the receptors are located on projection neurons to the striatum, a lesion of these neurons should result in a decrease in D4 receptor number. Adult Sprague-Dawley rats recieved infusions of ibotenic acid or vehicle into the medial prefrontal cortex. This region projects to the nucleus accumbens (core and shell). Rats were killed 6 weeks later. Brain sections (20 µm) were preincubated and then incubated with 1 nM [^{3}H]YM 09151-2. Adjacent sections were preincubated with 200 µM Gpp(NH)p and incubated with 6 nM [^{3}H]Raclopride. (+)-Butaclomal (10 µM) was used to determine non-specific binding in both assays. Mean optical density in the dorsolateral (DLS), ventromedial striatum (VMS), nucleus accumbens (NAC), NAC-core and NAC-shell were converted to fm/mg protein. Specific [^{3}H]raclopride binding was subtracted from specific [^{3}H]YM 09151-2 binding to estimate putative D4 receptor density. There was no effect of lesion for either ligand or for the calculated D4 receptor density in any region. These results suggest that D4 receptors are not located on cortico-striatal neurons projecting from medial prefrontal cortex to nucleus accumbens (core/shell). The limitations of using [^{3}H]YM 09151-2 minus [^{3}H]raclopride subtraction technique to measure D4 receptors will be discussed.

835.18

NEONATAL VENTRAL HIPPOCAMPAL DAMAGE CHANGES CORTICOSTERONE AND CENTRAL DOPAMINERGIC RESPONSES TO ACUTE FOOTSHOCK. S.J. Chrapusta, B.K. Lipska, M.F. Egan, R.J. Wyatt*, G.K. Wood and D.R. Weinberger. NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032.

Neonatal ibotenic acid ventral hippocampal (VH) damage enhances behaviors linked to dopamine (DA) system(s) in Sprague-Dawley rats, e.g. acute stress-induced locomotor activity, stereotypic and locomotor responses to DA agonists, etc. The VH-lesioned rats demonstrate an enhanced haloperidol challenge-induced DA release following chronic haloperidol treatment. However, they respond to chronic saline injections with a reduction of DA release in brain regions implicated in stress. We used continuous footshock (0.45 mA for 0, 20, and 60 min) to study the effects of this lesion on DA responses to acute stress in frontal cortex (FC), nucleus accumbens (NA), and striatum (ST). As an index of DA release, regional 3-methoxytyramine levels 10 min after pargyline hydrochloride injection (75 mg/kg, i.p.) were compared in microwave-fixed brains of the VH-lesioned and sham rats. Serum corticosterone (CORT) was measured as a peripheral index of stress. Basal DA release was attenuated in the NA but not in the FC or ST in the VH-lesioned rats. Footshock increased ST and NA DA release in the lesioned but not sham rats at both 20 and 60 min. In the FC, footshock-induced increases in DA release were found in both VH-lesioned and sham rats. The FC DA release normalized towards the end of the footshock session in the sham rats, but not in their VH-lesioned counterparts. Basal CORT levels were similar in the VH-lesioned and sham rats. Footshock increased CORT levels in both VH-lesioned and sham rats, but the patterns differed in that there was no attenuation over time in the VH-lesioned rats. Our observations indicate that the VH lesion interferes with the homeostatic systems involved in stress management in the rat, and delays adaptation to acute stress, which may potentiate detrimental effects of such event(s).

835.19

DOPAMINE RECEPTOR CHANGES IN RATS WITH NEONATAL VENTRAL HIPPOCAMPAL LESION ARE ONLY OBSERVED AFTER CHRONIC STRESS

G.K. Wood, B.K. Lipska, A.M. Murray, M.E. Knable, L.B. Bigelow*, D.R. Weinberger. Clinical Brain Disorder Branch, IRP, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032.

Neonatal exocitotoxic lesions of the rat ventral hippocampus (VH) produce postpubertal emergence of motoric hyperactivity to dopamine-related behavioral stressors. The accumulation of 3-methoxytyramine was reduced in the nucleus accumbens (NAC), striatum (STR) and medial prefrontal cortex (MPFC) in chronically stressed lesioned but not sham-operated rats. We now determined if stress-induced changes are associated with alterations of dopamine receptors in the MPFC, NAC and STR. On postnatal day 7 (PD7), the VH was lesioned with ibotenic acid. To test for age effects at baseline, rats were sacrificed at PD35 or PD56. In another group rats were stressed (daily saline injections for 3 weeks from PD35 to PD56) or untreated. Brain sections for autoradiography were incubated with [3H]raclopride (6.0nM), [3H]YM-09151-2 (YM, 1nM) or [3H]SCH-23390 (3nM). Raclopride and YM binding in the STR and raclopride binding in the MPFC decreased with age in all rats. At baseline, there was no difference between sham and lesioned rats at any age. Stress significantly increased the binding of raclopride in MPFC and YM in STR in lesioned but not in sham rats. Stressed lesioned rats had higher MPFC SCH23390 binding than stressed sham rats. The data suggest that previously reported behavioral hyperresponsiveness to acute stress in neonatally VH lesioned rats is not produced by increased D1, D2/D3 receptor levels. However, chronic stress upregulates D1 and D2/D3 levels in lesioned but not in control rats.

835.20

ALTERED INDUCTION PATTERNS OF c-FOS mRNA AFTER CLOZAPINE AND HALOPERIDOL IN RATS WITH NEONATAL HIPPOCAMPAL LESIONS. B. K. Lipska*, S. M. Lillrank, G. K. Wood, D. R. Weinberger. Clinical Brain Disorders Branch, IRP, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032.

Previous studies have indicated that neonatal (postnatal day 7) ibotenic acid lesions of the ventral hippocampus in the rat can be useful in modeling some aspects of schizophrenia. This developmental lesion results in postpubertal emergence of multiple abnormalities suggesting altered dopamine responsivity to environmental and pharmacological stimuli that can be ameliorated with antipsychotic drugs, clozapine (CLOZ) and haloperidol (HAL). In the present study we examined the effects of acute (i.p.) and chronic (via osmotic minipumps, 28 d) treatment with vehicle, CLOZ and HAL on c-fos expression in infralimbic region of medial prefrontal cortex (IF), dorsolateral (DLS) and ventromedial (VMS) striatum, and nucleus accumbens (NAC) in rats with neonatal hippocampal lesions. The effects of acute injections were drug-, lesion- and region-specific. In controls, acute HAL (0.2 mg/kg) increased c-fos mRNA by 30% (IF), 118% (DLS), 71% (VMS), 39% (NAC), $p<0.05$ vs vehicle. The response of lesioned rats to HAL was attenuated but significantly increased over vehicle, except for IF. Lesioned HAL-treated rats had significantly reduced c-fos mRNA in DLS, VMS and NAC, but not in IF, as compared to HAL-treated controls. In contrast, acutely CLOZ-treated lesioned rats showed reduced c-fos mRNA in IF and increased c-fos in VMS and NAC as compared with CLOZ-treated controls. There were no significant effects of chronic treatment on c-fos mRNA suggesting tolerance to both drugs. These data indicate that this developmental lesion differentially alters regionally-specific c-fos mRNA response to acutely administered neuroleptics.

835.21

EXPRESSION OF LIMBIC SYSTEM-ASSOCIATED PROTEIN (LAMP) IN RATS WITH NEONATAL EXITOTOXIC HIPPOCAMPAL LESIONS.

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We have previously demonstrated that rats with neonatal (day 7 after birth, PD7) ibotenic acid damage in the ventral hippocampus (VH) express postpubertally (PD56) a variety of abnormal behaviors related to mesolimbic and nigrostriatal dopaminergic and prefrontocortical dysfunction. We have hypothetized that the behavioral abnormalities elicited in this model might have resulted from an interaction of an early VH lesion with subsequent developmental maturation and with the development of anomalous limbic circuitry. LAMP is expressed preferentially by neurons in limbic structures or in neurons that receive direct projections from structures of the limbic system. It was shown previously that there is a critical period for cortical neurons to express LAMP. We studied the expression of the rat LAMP mRNA using in situ hybridization technique in adult rats with neonatal VH lesions. LAMP was expressed in all rats in the cingulate,- medial prefrontal and piriform cortex, nucleus accumbens, medial and lateral septal nuclei and claustrum. In adults there were no differences in LAMP expression between sham operated and neonatally lesioned animals in terms of optical density mesurements. These data support earlier studies indicating that the LAMP mRNA is expressed predominantly in the limbic regions. It also shows that the developmental VH lesion at PD7 does not permanently change the expression of LAMP mRNA in adult rats. We are currently examining LAMP mRNA expression in shorter time periods post lesion.

835.22

AMPA SPLICE VARIANT EXPRESSION IN MEDIAL TEMPORAL CORTEX IN SCHIZOPHRENIA AND CONTROLS . J. T. Noga,* S. E. Bachus, T. M. Hyde, M. M. Herman, D. R. Weinberger, J. E. Kleinman. NIMH, DIRP, Clinical Brain Disorders Branch, NIMH Neuroscience Center at St. Elizabeth's Hospital, Washington, DC 20037

The neurodevelopmental hypothesis of schizophrenia is supported by cortical cytoarchitectural abnormalities and volume decreases (localized and global) in the absence of gliosis. Receptor density and gene expression abnormalities in glutamatergic (and other) neurotransmitter systems have also been reported. These abnormalities have been concentrated primarily in prefrontal, medial temporal, and striatal structures. In this study we tested neurodevelopmental aspects of receptor gene expression in schizophrenia by examining the relative presence of ontogenetically regulated flip and flop isoforms of AMPA receptor mRNA in medial temporal structures of postmortem brains from adult schizophrenics (n=6, mean age 56.8 ± 16.3 years), normal controls (n=6, mean age 50.8±12.2), and medicated nonschizophrenic psychiatric controls (n=8, 46.8±16.7) using in situ hybridization of [^{35}S]-labelled oligonucleotide mRNA probes with 20 µm thin frozen sections. ANOVA results of densitometric analysis revealed a main effect of isoform type consistent with previous literature reports, namely that the flip isoform predominated in the regions measured (entorhinal cortex, deep intermediate/lateral ($p<.0002$) and superficial intermediate/lateral ($p<.07$), and deep ($p<.0002$) and superficial ($p<.0002$) sulcal subregions; dentate gyrus ($p<.0002$); and subiculum ($p<.0002$)) in all diagnostic groups. The schizophrenia group did not differ from the other 2 diagnostic groups in the density of flip or flop isoforms or in their ratio ($p>.2$). We conclude that there is no apparent abnormality in the expression of GluR3 flip or flop isoforms in this sample of schizophrenia v. control subjects. These results do not support the presence of an aberration in GluR3 splice variant ontogeny in schizophrenia. GluR1,2, and 4 isoforms are being studied as well in this group of brains and will be reported.

835.23

rCBF PATTERN DIFFERENCES BETWEEN SCHIZOPHRENIC PATIENTS WITH AND WITHOUT DEFICIT SYMPTOMS. W.T. Carpenter, Jr.*, A.C. Lahti, H.H. Holcomb, P.J. Caudill, M. Zhao, D.R. Medoff, C.A. Tamminga. MPRC, University of Maryland School of Medicine, P.O. Box 21247, Baltimore, MD 21228

We previously reported that schizophrenic patients with deficit symptoms (primary negative symptoms) were distinguished from non-deficit patients and normals in a PET/FDG study by reduced metabolism in thalamus and in frontal and parietal cortex (Tamminga et al, 1992). We now report $H_2{}^{15}O$ PET data on drug-free schizophrenic patients with (N=5) and without (N=5) deficit symptoms and normals (N=12). Rest, sensory-motor control (SMC), and tone discrimination task scans were obtained on each subject, four of each. Task difficulty was manipulated to assure a comparable accuracy of 80% in each subject group. Statistical Parametric Maps (SPM 94 MRC Cyclotron Unit, Hammersmith Hospital) were created to contrast rCBF activity patterns between groups. Preliminary analyses show that while normal volunteers show physiologically appropriate patterns of activation, deficit and nondeficit patients have different patterns from normals at rest and during tasks. In normals, during the tone discrimination task, right middle frontal and anterior cingulate cortices are activated. In patients, deficit patients have reduced parieto-occipital flow at rest and reduced frontal cortex flow during the tone discrimination task, compared with nondeficit schizophrenic patients.

NEUROTOXINS IV

836.1

HIGH GLUCOCORTICOID LEVELS DECREASE SOME ANTIOXIDANT ENZYME ACTIVITIES IN THE ADULT RAT BRAIN. L.J. McIntosh,* K.E. Hong and R.M. Sapolsky. Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305

Due to its high oxygen consumption, the brain may be particularly vulnerable to oxidative damage and degeneration. Substances affecting neuronal oxidative homeostasis would therefore be expected to alter the ability of the brain to defend itself against oxidative damage. High levels of glucocorticoids (GCs), the adrenal hormones secreted in response to stress, can change metabolic capacity in various brain regions by decreasing energy uptake and elevating intracellular calcium, thereby altering intracellular oxidative homeostasis. We have recently shown that GCs enhance oxygen radical-associated neurotoxicity in vitro, supporting previous in vivo studies demonstrating that GCs potentiate neurodegeneration following oxidative insults (e.g. stroke, hypoglycemia, seizure). To investigate whether GCs affect the enzymatic component of intracellular oxidative defenses, we assayed Cu/Zn- and Mn-superoxide dismutase, catalase, and glutathione peroxidase in the livers and several brain regions of rats which had been either adrenalectomized (ADX) to remove circulating GCs, or supplemented with GCs to achieve high physiological levels. GCs altered enzyme activity in a pattern unique for each enzyme. For example, catalase activity in GC-treated rats was decreased 50% in liver and 66% in cortex, and did not change significantly in hippocampus and cerebellum as compared to ADX rats. Glutathione peroxidase activity decreased 50% in hippocampi of high GC animals, but was essentially unchanged in liver and cortex. Western blotting is being used to determine whether changes in activity are a genetic or epigenetic effect. Our results indicate that stress hormones may directly affect pathways involved in oxygen radical toxicity in adult brain, and imply that the GC potentiation of damage seen after oxidative insults is due in part to a decrease in the cellular enzymatic antioxidant defenses.

836.2

KETAMINE INHIBITS GLUTAMATE INDUCED NEURONAL DEATH. 1)Masanori YAMAUCHI, 1)Akiyoshi NAMIKI, 2)*Takahumi NINOMIYA 1)Dept. of Anesthesiology, Sapporo Medical University 2)Dept. of Anatomy Sect. I, Sapporo Medical Universit

We studied the effect of ketamine, non-competitive NMDA-receptor antagonist, on glutamate-induced neuronal death in cultured rat cortical neurons. After 7 days in culture, the neurons were exposed to 1mM of glutamate or both glutamate and 10-1000μM of ketamine. After 24-hr. exposure, they were stained with a monoclonal anti-microtuble-associated protein 2 antibody. The number of surviving cultured neurons was decreased by a exposure of glutamate. Concomitant exposure of glutamate and ketamine did not decrease the number of neurons in a concentration-dependent manner. Ketamine likely protects the glutamate-induced neuronal death.

836.3

TROLOX INHIBITS CELL DEATH FOLLOWING COMBINED OXYGEN AND GLUCOSE DEPRIVATION IN MIXED NEURONAL/GLIAL CORTICAL CULTURES. A.W. Probert*, F.W. Marcoux and P.A. Boxer. Parke-Davis Pharmaceutical Res., Div. of Warner-Lambert Co., Ann Arbor, MI 48105.

Exposure of mixed neuronal/glial cortical cultures to increasing intervals of combined oxygen [1%] and glucose [1 mM] deprivation (COGD) results in a gradual rise in neuronal cell death measured 18-22 hours post COGD. Injury-onset (LDH release) first appears following 135 to 150 minutes of COGD, climaxing after 240 to 255 minutes of deprivation. Treatment with the vitamin E analog, Trolox, a free radical scavenger, increased the deprivation intervals required to initiate COGD-induced neuronal death. This postponement of injury-onset was concentration dependent, delaying the induction of cell death by 30 to 60 minutes for Trolox concentrations of 10 μM to 1000 μM. Trolox did not alter the deprivation interval required to achieve maximal injury. Co-treatment with Trolox (100 μM) and the NMDA competitive antagonist, CPP (3(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid, 100 μM), produced a synergistic effect, prolonging deprivation intervals required to induce both injury-onset and maximal death. In both cases, deprivation intervals required to override CPP's protection were increased by 30 to 45 minutes with Trolox co-treatment. Results suggest free radical formation may contribute to the early stages of neuronal injury initiated by oxygen and glucose deprivation.

836.4

CALBINDIN-D28k IS PRESENT IN MIDBRAIN DOPAMINERGIC NEURONS THAT ARE LESS VULNERABLE TO MPTP-INDUCED DEGENERATION. C.-L. Liang, C.M. Sinton*, P.K. Sonsalla, and D.C. German. Dept Psychiat, UT Southwestern Med Cntr, Dallas, TX 75235-9070, and Dept Neurol, UMDNJ RW Johnson Med Sch, Piscataway, NJ 08854.

The calcium-binding protein, calbindin-D28k (CALB), is in nerve cells in midbrain regions where dopaminergic (DA) neurons reside that are less vulnerable to degeneration in Parkinson's disease (PD), and in an animal model of PD (the MPTP-treated mouse). In order to determine whether the CALB-containing DA neurons are less vulnerable to degeneration in the MPTP-treated mouse, immunohistochemical staining and computer mapping techniques were employed. Male C57BL/6 mice were treated with saline or MPTP (4x20 mg/kg on Day 1, and 3x20 mg/kg on Day 3) and sacrificed on Day 9. Ten μm coronal sections were cut through the midbrain, and sections were double immunostained for CALB and tyrosine hydroxylase. The locations of all TH, and TH+CALB cells in a midbrain region were mapped. In animals treated with MPTP (n=5), compared to controls (n=3), there was a 72% reduction in TH-containing cells ($p < 0.001$), but only a 30% reduction in TH+CALB-containing cells ($p < 0.02$). These data support the hypothesis that DA cells that contain CALB are less vulnerable to MPTP-induced degeneration than DA cells lacking CALB.

836.5

G_{M1} GANGLIOSIDE INFUSION PRODUCES INCREASED GFAP IMMUNOREACTIVITY IN THE ABSENCE OF NEUROPATHOLOGY.
G. P. H. Ballough*,[2], P. R. Patel[2], J. P. Makowski[2], M. A. O'Connor[2], F. J. Cann, C. D. Smith[1] and M. G. Filbert[1]. [1]Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, Edgewood Area - Aberdeen Proving Ground, MD 21010-5425. [2]Department of Biology, La Salle University, Philadelphia, PA, 19141.

Glial fibrillary acidic protein (GFAP) immunodetection, as a marker of reactive astrocytosis, has become a mainstay of modern neurotoxicology. It is well established that increased GFAP immunostaining is a sensitive, early index of neurotoxic insult. As part of an ongoing study to evaluate the effectiveness of G_{M1} administration to ameliorate brain damage resulting from soman-induced seizures, we observed an apparent induction of reactive astrocytosis in the absence of neuropathology. Male Sprague-Dawley rats received G_{M1} (5 mg/kg/day, for 5.0 ± 0.5 days) through a permanent cannula implanted intracerebroventricularly (i.c.v.) and connected to an osmotic minipump. Controls received saline infusions or were sham operated. Rats from each of these groups received either soman (83 µg/kg, i.m.) or saline injections 4.0 ± 0.5 days following initiation of G_{M1} infusions. GFAP immunostained brain sections were assessed both visually and using optical density image analysis. These results demonstrate a significant and marked increase in GFAP immunostaining in the G_{M1} infused non-soman group compared to the other groups; this finding was not associated with neuropathology (determined on H&E-stained serial sections). Elevations in GFAP were most pronounced in the hippocampi of these non-seizing animals. This study provides new information concerning GFAP as a marker for brain damage, and indicates the possibility that astrocytic reactions may be uncoupled from neuropathology.

836.6

CHRONIC G_{M1} GANGLIOSIDE ADMINISTRATION PROTECTS AGAINST SOMAN-INDUCED SEIZURE-RELATED BRAIN DAMAGE.
M. G. Filbert, F. J. Cann, C. D. Smith, C. E. Kling, J. S. Forster, J. S. Graham, B. E. Hackley* and G. P. H. Ballough^. Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, Edgewood Area - Aberdeen Proving Ground, MD 21010. ^Department of Biology, La Salle University, Philadelphia, PA, 19141.

Soman (pinacolylmethylphosphonoflouridate) is an irreversible acetylcholinesterase (AChE) inhibitor which produces seizure-related brain damage in mammals. In the present study, we examined possible ameliorative effects of chronic G_{M1} administration on brain damage resulting from soman administration. Male Sprague-Dawley rats received G_{M1} (5 mg/kg/day, for 5.0 ± 0.5 days) through a permanent cannula implanted intracerebroventricularly (i.c.v.) and connected to an osmotic minipump. Controls received saline infusions or were sham operated. Rats from each of these groups received either soman (83 µg/kg, i.m.) or saline injections 4.0 ± 0.5 days following initiation of G_{M1} infusions. Damage was assessed using H&E and cresyl violet (CV) histochemistry as well as glial fibrillary acidic protein (GFAP) and microtubule associated protein-2 (MAP2) immunohistochemistry. These results show that two mechanisms of neuroprotection were produced by chronic GM_1 infusion: First, GM_1 administration interfered with the development of seizures and status epilepticus; in these situations, no brain damage was observed. Secondly, brain damage resulting from sustained seizures was significantly attenuated in GM_1 treated rats.

836.7

INHIBITION OF BRAIN NITRIC OXIDE SYNTHASE BY S-METHYL-ISOTHIOUREA
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S-methyl-isothiourea (SMT) is a relatively selective inhibitor of inducible nitric oxide (NO) synthase (iNOS) that improves survival in a rodent model of septic shock (PNAS, 91, 12472, 1994). Because, iNOS induction may contribute to some forms of brain injury, including ischemic damage (Iadecola et al., this meeting), it would be important to determine whether SMT could be used as a selective iNOS inhibitor in brain. Calcium-dependent (cNOS) and independent (iNOS) NOS activities were measured in brain homogenates using the citrulline assay. iNOS was induced in the rat cerebral cortex by thermal lesions and in systemic organs by i.p. administration of lipopolysaccharide antigen (LPS). SMT (0.01-1000µM) attenuated cNOS activity of brain homogenates in a dose-dependent manner (EC_{50}=0.9µM). SMT was nearly as potent as nitro-L-arginine (L-NA; EC_{50}=0.7) and was, respectively, 11 and 814 times more potent than monomethyl-L-arginine (L-NMMA) and aminoguanidine (AG). SMT attenuated iNOS activity in lung homogenates of LPS-treated rats (EC_{50}=0.09) and was 12, 115, and 1152 times more potent than L-NMMA, L-NA and AG, respectively. We then evaluated the ability of SMT to inhibit cNOS and iNOS in brain *in vivo*. SMT (1-100 mg/kg; i.p.; n=4 per dose) inhibited brain cNOS dose-dependently and up to 34±4% (100 mg/kg). Higher SMT doses (500, 1000mg/kg) produced seizures and death. In contrast, L-NA (40 mg/kg; i.p.) inhibited cNOS by 75±2% (n=4). SMT (100 mg/kg) failed to attenuate iNOS activity in homogenates of thermal brain lesions (p>0.05 from vehicle). Thus, the *in vitro* data suggest that SMT is a more potent inhibitor of iNOS than L-NA, L-NMMA or AG. However, *in vivo*, SMT inhibits only brain cNOS and less effectively than L-NA. We conclude that SMT is a potent iNOS inhibitor *in vitro*. However, its lack of effectiveness *in vivo* limits its usefulness in models of iNOS-dependent brain injury. (Supported by AHA and NIH)

836.8

LOW DOSE SELEGILINE TREATMENT MEDIATES NEURONAL RESCUE IN RAT PERIPHERAL SYMPATHETIC NERVOUS SYSTEM. T. Salonen, A. Haapalinna #, E. Heinonen #, J. Suhonen + and A. Hervonen *, Univ. of Tampere, School of Public Health, Dept. of Gerontology, FIN-33101 Tampere, FINLAND; # ORION Corp., FARMOS Research, Dept. of Neurological Products, FIN-20101 Turku, FINLAND; + The Salk Institute, La Jolla, CA 92093, USA

Selegiline (SEL) is a selective and irreversible monoamine oxidase type B (MAO-B) inhibitor. It has been shown to protect against toxic effects of aminergic neurotoxins. Recent animal studies have suggested that low doses of SEL has also neuronal rescue and antiapoptotic effects after neuronal damage or withdrawal of trophic factors, respectively. The purpose of this study was to determine the effect of SEL on the recovery of adrenergic nerve fibres after 6-OHDA-induced degeneration. The animals were treated with 6-OHDA (50 mg/kg i.p.) and 24 hours after that the treatments with SEL (0.03, 0.1 and 1 mg/kg s.c.) or distilled water were started and then continued daily. The animals were killed one or two weeks after the 6-OHDA administration and the SCG's and SMG's were dissected. The treatment with 6-OHDA had significantly decreased the number and length of the nerve fibres in the SMG. Subchronic SEL treatment had enhanced recovery rate and there was a statistically significant difference between SEL 0.1 mg/kg group and 6-OHDA treated control group in SMG innervation at two week time point. The degenerative effect of 6-OHDA on the ganglion cell somata was detected in this experiment similar to the previous experiments. The somata of the cells seemed to prefer the highest dose of SEL for the fastest recovery.

836.9

THE NEUROTOXIN MPTP INCREASES CALBINDIN-D_{28K} LEVELS IN MOUSE MIDBRAIN DOPAMINERGIC NEURONS.
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The calcium-binding protein calbindin-D_{28k} (CALB) has been localized in high concentration in several neuronal populations within the CNS and is believed to act as an intracellular calcium (Ca^{2+}) buffer. There has been much interest and speculation concerning its potential neuroprotective function. However, there is little direct evidence linking CALB content of individual neurons to Ca^{2+} buffering ability, resistance to Ca^{2+}-mediated excitotoxicity, or vulnerability to Ca^{2+}-mediated degeneration. It is necessary to demonstrate these relationships on a cellular level so that more definitive conclusions can be made. We have utilized immunocytochemical and Western blot techniques to determine whether cellular CALB content is altered in the nucleus A10 dopaminergic region of the midbrain following administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Our data demonstrate a significant increase in the CALB content of nucleus A10 neurons (up to 227 ± 23% above control) 3 and 6 hours after MPTP treatment. CALB elevation demonstrated both time and dosage dependency as 6 hour groups exhibited larger increases than 3 hour groups, and a 60mg/kg dosage induced a larger increase than a 20mg/kg dosage. The time course of CALB elevation is consistent with upregulation of gene transcription. These data support the hypotheses that MPTP is neurotoxic by causing increases in intracellular Ca^{2+} and that increased CALB in midbrain dopaminergic neurons is a protective response to elevated intracellular free Ca^{2+}.

836.10

K252A, K252B AND STAUROSPORINE INCREASE HIPPOCAMPAL NEURON SURVIVAL AND IMPROVE WATER MAZE PERFORMANCE AFTER KAINATE LESION.
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Staurosporine, K252a and K252b are low molecular weight alkaloids which act as protein kinase inhibitors that antagonize the effects of neurotrophic factors at high concentrations (µM); but have neuroprotective activities at low concentrations (fm-nM). We found that systemic administration of K252a, K252b or staurosporine (1µg/kg) significantly protected rat hippocampal neurons against both unilateral and bilateral hippocampal kainic acid (KA) lesions. Twenty-four hr pretreatment with K252b or staurosporine significantly protected CA3 neurons against unilateral KA lesion ($p<0.05$). Chronic administration of these agents for 2 mos further increased CA3 neuron survival after KA lesion by 1220% and 720%, respectively. Seven day pretreatment with either K252a, K252b, or staurosporine led to a significant increase in CA1, CA3 and hilar neuron survival after bilateral KA lesion ($p<0.05$). Moreover, these compounds improved either learning or performance on the Morris water maze. In addition, we found with western blots that 24 hr treatment with K252b or staurosporine led to a distinct change in excitatory amino acid receptor levels in vitro and in vivo, such that the NMDA receptor subunit NR1 was decreased and the AMPA receptor subunit GluR1 was increased. The ability of these lipophilic alkaloids to be given systemically and have a central neuroprotective effect against excitotoxic/metabolic insults may have profound implications for treatment of several neurodegenerative disorders.

836.11

***IN VITRO* STUDIES ON ANTIOXIDANT PROPERTIES OF METALLOTHIONEIN AND ITS POSSIBLE ROLE IN NEUROPROTECTION.**
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Metallothionein (MT) is a low molecular weight (6-7 kd) metal binding protein found in biological systems. Its role as an antioxidant is still unclear. The present study was designed to examine the *in vitro* antioxidant properties of commercially available MT and to compare with MT isolated from mouse brain. Different concentrations of MT I and II have been used to determine their capacity to scavenge $O_2^{\cdot-}$ generated from the xanthine/xanthine oxidase (X/XO) system that is based on the i aaaaaaassnhibition of nitrite formation. Other -SH containing molecules e.g. cysteine and GSH, were also used to assess superoxide scavenging properties and to compare with MT. In addition, we have also isolated MT-like protein from mouse brain that inhibits nitrite formation. The results show the concentration dependent $O_2^{\cdot-}$ scavenging properties of MT and MT II has a three- fold higher capacity than MT I. The order of superoxide scavenging properties was found as follows: MTII> CYS> GSH> MTI. A positive control experiment was run with pure SOD that is known to scavenge $O_2^{\cdot-}$ to confirm the X/XO system. The reduction of -SH groups could be due to their oxidation by $O_2^{\cdot-}$. The -SH groups of brain protein fraction were detected qualitatively by dot blotting using a DIG protein kit from Boehringer. Results from this experiment show that a substantial number of -SH groups may be involved in scavenging $O_2^{\cdot-}$. In conclusion, the present study suggests that MTs and MT-like proteins isolated from mouse brain scavenge the superoxide radical *in vitro*. Further, *in vivo* experiments are in progress to substantiate the antioxidant properties of metallothioneins.

836.12

PROTECTIVE EFFECTS ON NEURONAL CELLS BY ANTIOXIDATIVE AGENTS OF MICROBIAL ORIGIN.
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A number of neuronal disorders such as brain ischemic injury, Alzheimer's disease and amyotrophic lateral sclerosis (ALS) have proven to be caused by oxygen free radicals. In the course of our screening for antioxidant microbial substances to prevent or ameliorate these diseases using an *in vitro* glutamate-toxicity model, we isolated several potent microbial metabolites (Shin-ya *et al.*, *Tetrahedron Lett.*, **34**: 4943-4944 (1993)). Here, we report the effects of those compounds on neuronal survival.

Cultured rat hippocampal neurons maintained in serum free media were exposed to L-glutamate and other glutamate receptor agonists with or without antioxidants of microbial origin. Some of those antioxidants effectively prevented neuronal cell death by lowering levels of intracellular oxygen radicals with concentrations around 20-200 nM, whereas vitamin E showed little effect with concentration 50 mM. These compounds also protected primary hippocampal neurons and PC12 cells from apoptosis induced by trophic factor deprivation. Since antioxidants were reported to protect against amyloid β toxicity (Behl *et al.*, *Cell*, 77, 817-827 (1994)), we also assessed the activities of those compounds against Aβ toxicity in primary hippocampal neurons and Aβ induced-decrease of MTT reduction in PC12 cells. NADPH oxidase inhibitors like diphenylene iodonium were not effective; only vitamin E type antioxidants protected against Aβ toxicity in our system. Taken together, these findings confirmed the importance of oxygen radicals in neuronal cell death and the usefulness of antioxidant application to neuronal cell survival.

836.13

PROTECTION OF NEURONAL VIABILITY AND INHIBITION OF LIPID HYDROPEROXIDE FORMATION BY TIRILAZAD MESYLATE.
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The 21-aminosteroid, tirilazad mesylate (U-74006F), has demonstrated neuroprotection in a variety of CNS injury models, most likely, through inhibition of lipid peroxidation. In the present study, the compound has been shown to improve neuronal viability in an *in vitro* model of iron-induced lipid peroxidation. Utilizing HPLC-chemiluminescent technology, a correlation has been established between U-74006F protection of neuronal viability and a decrease in lipid hydroperoxide (LOOH) formation. Exposure of murine spinal cord neuronal cultures to 30 μM ferrous ammonium sulfate (FAS) for 20 minutes resulted in an increase in phosphatidylcholine, phosphatidylethanolamine, and free fatty acid LOOH formation. LOOH levels increased 6, 50 and 28 fold, respectively. Prior to iron insult, cell cultures were treated for 60 minutes with U-74006F in half-log concentrations ranging from 0.3 to 30 μM. LOOH levels were reduced significantly in a concentration dependent manner. A 30 μM concentration of U-74006F essentially decreased LOOH levels to control levels and maintained cellular viability near 100%. Correlation between cell death and LOOH formation provides further confirmation of the mechanism of action of U-74006F.

836.14

DIFFERENTIAL EXPRESSION OF CALMODULIN AND CALMODULIN-BINDING PROTEINS AFTER KAINIC ACID ADMINISTRATION TO THE MOUSE.
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An increase in intracellular calcium has been repeatedly involved in the development of neuronal damage. Calmodulin (CaM), a major calcium binding protein in mammalian brain, is expressed by three different genes (CaM I, CaM II and CaM III) that encode an identical protein. Nevertheless, little is known about the regulation of these genes or about their role in calcium mediated neuronal damage. In turn, CaM mediates its action through its union to different CaM-binding proteins (CaMBP) and among them the calcium-CaM dependent protein kinase II (CaMKII) and the phosphatase calcineurin are highly enriched in the brain.

We studied the expression of the three CaM genes and the two CaMBP above mentioned in the mouse brain after intraperitoneal administration of kainic acid (KA) by means of *in situ* hybridization histochemistry, in several brain regions and at different times of the administration (5 h, 24 h, 2 d and 4 d). The role of CaM and CaMBP following this neurotoxic insult has not been characterized until now.

In general, our results show that KA treatment induced a transient increase in CaM I mRNA hybridization signal in mouse brain 5 h after KA. On the contrary, KA treatment produced a decrease in CaM II mRNA hybridization signal 24 h following the administration in most brain regions considered, while CaM III mRNA signal was mostly unaffected. In addition, a decrease in CaMKII mRNA hybridization signal was observed in regions of cerebral cortex and hippocampus, effect already observed at 5 h of KA and still present in some regions 4 d after. Mainly, these results suggest a differential response of CaM genes to neurotoxic insult.

836.15

EFFECTS OF GLUTAMATE-INTOXICATION ON VOLTAGE- AND TRANSMITTER-GATED ION CURRENTS IN CULTURED CORTICAL NEURONS:COMPARISON WITH DIZOCILPINE-TREATED CULTURES
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Voltage- and transmitter-induced currents were investigated in cultured cortical neurons of fetal rats after an exposure to glutamate (10 and 100 μM for 20 h) using the whole-cell configuration of the patch-clamp technique. Voltage-gated currents were induced by voltage steps from -60 to +30 mV; transmitter-gated currents were induced by pulse applications of glutamate (500 μM), NMDA (100 μM), AMPA (50 μM), kainate (1 mM) or GABA (10 μM). The results were compared to those of cultures co-exposed to glutamate and dizocilpine (1 μM).

Exposure to glutamate (100 μM) destroyed 69±4% of the neurons. Surviving cells showed the following changes: The capacity of the cell membrane as a parameter for cell size was reduced. The sodium peak current was reduced from a mean of -3095 pA to -380 pA; the potassium steady state current decreased from a mean of 1857 pA to 614 pA. The relative GABA-induced current was reduced from 25 pA/pF to 9.7 pA/pF. All these effects were significant ($p<0.05$).

These changes induced by glutamate exposure were abolished by co-incubation with the NMDA-antagonist dizocilpine (1μM).

The currents induced by pulse applications of glutamate, NMDA, AMPA and kainate were not significantly different between glutamate-intoxicated and control cells.

These results demonstrate that glutamate intoxication selectively influences certain functional parameters of cultured neurons, and that these changes can be counteracted by the application of a non-competitive NMDA-antagonist.

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836.16

KAINATE AND AMPA RECEPTORS AND DOPAMINE RELEASE IN EXPERIMENTAL HEPATIC ENCEPHALOPATHY.
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Salient features of hepatic encephalopathy (HE) include extrapyramidal symptoms, such as rigidity and tremor. Our working hypothesis was that these symptoms may be associated with an impaired glutamatergic regulation of dopamine release in the striatum. We thus investigated how the functions of kainate and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors and the release of dopamine are altered in the frontal cerebral cortex and striatum in experimental HE induced in male Wistar rats with repeated intraperitoneal injections (300 mg/kg) of a hepatotoxin thioacetamide. In HE, the bindings of both AMPA and kainate to synaptic membranes were increased in the frontal cerebral cortex. The release of dopamine evoked by kainate was correspondingly enhanced. In the striatum the binding of kainate was reduced and the kainate-evoked release of dopamine depressed in HE. Of the binding parameters, K_D was generally affected less than B_{max}, e.g., B_{max} for AMPA increased twofold in the frontal cerebral cortex and B_{max} for kainate decreased to the same degree in the striatum, whereas no significant changes were discernible in K_D. The mechanism of dopamine release was not itself compromised in HE, as indicated by persisting potassium stimulation of the release in both brain regions studied. The present results demonstrate that the glutamatergic regulation of brain dopaminergic systems is indeed impaired in HE and the neurological symptoms are thus likely to stem from this defect.

836.17

EFFECT OF NITRIC OXIDE ON STP IN CA1 AND CA3 AREA OF RAT HIPPOCAMPAL SLICES CHRONICAL EXPOSURE TO LEAD.

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Neonatal Wistar rats were exposed to lead from parturition to weaning via the milk of dams drinking 0.2% lead acetate solution. Previous studies in our lab had shown that lead-exposed rats led to a significant reduction on STP in CA1 area and a significant increase on STP in CA3 area (Neursoi.Abst. 885.18, 1993). IN this study we tested the effect of the NO-generating compound Sodium Nitroprusside (SNP) on STP by recording field excitatory postsynaptic potentials (EPSPs) extracellularly in hippocampal slices from control and lead-exposed rats. By bath application of 1mM SNP, STP in CA1 area of control rats were significantly increased in magnitude from 187±39% (n=8) to 270±101% (n=8, $p<0.05$) and that in lead-exposed rats were significantly increased from 129±14% (n=8) to 297±104% (n=8, $p<0.01$). While in CA3 area of control rats, by bath application 1 mM SNP, the magnitude of STP were significantly increased from 119±18% (n=8) to 146±27% (n=8, $p<0.05$) and that in lead- exposed rats were not significantly increased from 187±35% (n=8) to 201±78% (n=8). This data suggest that NO may be a messenger molecule both in CA1 and CA3 area. There are effect of lead on the retrograde messenger system in postsynaptic neurons in CA1 area and on the inhibitory interneuron in CA3 area. Supported by NNSFC (DYR).

836.18

***IN VIVO* VERSUS *IN VITRO* EXPRESSION OF RAT INDUCIBLE NITRIC OXIDE SYNTHASE BY ACTIVATED MICROGLIA AND REACTIVE ASTROCYTES.** C.G. Pilapil*, C.E. Nolan, M.A. Collins, R. Tracey, and R.B. Nelson, Dept. of Neuroscience, Pfizer Central Res., Groton, CT.

The two endogenous CNS inflammatory response cells are microglia and reactive astrocytes. Both of these inflammatory response cells from rat can be stimulated *in vitro* with non-specific pro-inflammatory factors (opsonized zymosan or lipopolysaccharide) to release micromolar quantities of nitric oxide (NO), synthesized by an inducible nitric oxide synthase (iNOS). We have also determined in co-culture bioassays that microglia and astrocytes stimulated with pro-inflammatory factors kill adjacent hippocampal neurons, an effect completely abrogated by NOS inhibitors. Little is known about the *in vivo* regulation of iNOS expression in microglia or astrocytes. To begin characterizing the circumstances under which iNOS is expressed in rat brain, we induced unilateral glial reactivity in area CA3 of the rat hippocampal formation via kainic acid administration in the lateral ventricle. We found that by 2 days post-lesion, iNOS immunoreactivity (based on 2 different iNOS-specific antibodies) appeared in cells around the lesion site having reactive astrocyte morphology. Cells of the same size, location, and morphology stained immuno-positive for the astrocyte marker GFAP, and for NADPH-diaphorase activity (a non-specific NOS marker). Cresyl violet staining verified the near-complete destruction of area CA3 pyramidal cells on the injected side. The staining intensity of all 3 of the above markers was increased by 5 days post-lesion and later timepoints are being examined. In animals receiving only vehicle injection, the same 3 markers appeared by 2 days in cells of reactive astrocyte morphology along the cannula tract. Staining of OX-42, a marker for activated microglia, also greatly increased as a result of the kainate lesion, but only a few of the iNOS-positive cells had a morphology or size consistent with OX-42 staining. These results indicate that: 1) both astrocytes and microglia from rat can express iNOS *in vitro*; 2) differential iNOS expression by these cells can occur *in vivo*; and 3) rat brain injury induces predominantly astrocytic iNOS expression.

836.19

FREE RADICAL AND NON FREE RADICAL MEDIATED ALTERATIONS IN Ca^{2+} FLUX IN PC-12 CELLS. J.G.Strain, N.D.Jimenez, G.Cao* and J.A.Joseph. USDA-Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Previous research has suggested that the initial effects of cellular free radical neurotoxic insult involve large increases in intracellular Ca^{2+}. However, the exact role of oxidative stress on the various parameters involved in these increases has not been specified, especially in the case of low, non lethal concentrations of H_2O_2. Present experiments were carried out to examine these parameters in PC-12 cells exposed to 300 uM H_2O_2 for 30 minutes in growth medium alone or containing either nifidipine (L-type Ca^{2+} antag.), conotoxin (N-type antag.), trolox (Vitamin E analog), or *n*-tert-butyl-α-phenylnitrone (nitrone trapping agent, PBN). Fluorescent imaging was used to visualize intracellular Ca^{++} changes in individual Fura II-loaded cells. Baseline (pre-30 mM KCl) Ca^{++} levels were significantly increased by H_2O_2 treatment (200%), while the rise in free intracellular Ca^{++} following KCl stimulation was decreased (50%) and the cell's ability to clear the excess Ca^{++} (i.e., Ca^{2+} recovery time, Ca^{2+}RT) following depolarization was signifcantly increased. Nifidipine, conotoxin, trolox and PBN prevented the initial increase in Ca^{++}, and with the exception of conotoxin, antagonized the H_2O_2-induced decreases in Ca^{++} flux following KCl stimulation. Interestingly, not only were none of these agents effective in protecting against H_2O_2-induced decreases in the cell's ability to clear excess Ca^{++} following depolarization, all of these agents except PBN increased Ca^{2+}RT without H_2O_2 and in fact, acted synergistically with H_2O_2 on this parameter.

836.20

INDUCTION OF CELLULAR STRESS BY INHIBITION OF SUPEROXIDE DISMUTASE OR HEAT SHOCK ALTER UBIQUITIN CONJUGATION IN PC12 CELLS. S.J.Flann, P.W.Beesley* and C.C. Rider, School of Biological Sciences, Royal Holloway, University of London, Egham Hill, Egham, Surrey TW20 OEX, U.K.

Ubiquitin is a low molecular weight polypeptide which is commonly found conjugated to a wide range of target proteins via the ϵ-amino group of lysine. A major role of this conjugation is to target proteins for rapid degradation, although ubiquitination has other functions, as yet not clearly defined. Ubiquitin is a heat shock protein and its mRNA levels are elevated by a number of stress conditions. We have, therefore, studied the effect of cellular stress induced by inhibition of superoxide dismutase (SOD) or heat shock, on ubiquitin conjugates in differentiated PC12 cells. Elevated free radical levels were induced by inclusion of the SOD inhibitor diethyldithiocarbamic acid (DDTC) in the culture medium, at concentrations varying from 10^{-3} to 10^{-5} M. Ubiquitin conjugates were detected by immunodevelopment of Western blots using an anti-ubiquitin monoclonal antibody. Within 1 h the level of high molecular weight conjugates ($Mr > 60$ kD) is elevated at all inhibitor concentrations relative to control. At 10^{-5} M DDTC, these levels remain elevated for at least 4 h but return to control levels by 24 h. Little loss of cell viability, measured by dye exclusion, was observed. In contrast, at 10^{-3} M DDTC the conjugate levels are still elevated at 24 h, at which time there is marked loss of viability. A concomitant decrease in the level of a putative ubiquitin-histone conjugate was observed. Induction of sublethal stress by heat shock of PC12 cells for 3 h at 43°C, also caused a rapid marked elevation in the level of high molecular weight conjugates for up to 24 h, relative to control cells. These results show that ubiquitin-conjugation is a regulated process and can alter rapidly in response to conditions which induce stress in neurone-like cells.

836.21

THE ROLE OF HIPPOCAMPAL Cu, Zn-SUPEROXIDE DISMUTASE (SOD-1) IN KAINIC ACID-INDUCED NEURONAL DEGENERATION. H. C. Kim[1]*, S. J. Kim[2], G. Bing and J. S. Hong. [1]College of Pharmacy, Kangwon National University, Chuncheon, 200-701, KOREA, [2]Lab. of Chemoprevention, NCI/NIH, Bethesda, MD 20892 and LEN, NIEHS/NIH, Research Triangle Rark, NC 27709, USA.

We examined the immunocytochemical distribution of cytosolic Cu, Zn-superoxide dismutase (SOD-1), the expression of SOD-1 mRNA and the activities of SOD-1, glutathione peroxidase (GSHPx) and catalase (Cat) in the rat hippocampus after systemic injection of kainic acid (KA). Intense immunoreactivity of SOD-1 was seen in the pyramidal cell layer 3·5 days after injection of KA, while weak immunoreactivity for SOD-1 was observed in the pyramidal cell layer in control rats. The increased immunoreactivities and mRNA levels for SOD-1 were consistent with the enhanced enzyme activities of SOD-1 following KA. However, the increased activity of SOD-1 after KA treatment was not accompanied by adaptive rises in the activities of GSHPx and Cat. High levels of SOD-1, and low levels of GSHPx and Cat, may increased the intracellular accumulation of H_2O_2. This increased H_2O_2 might faciliate the lipid peroxidation of hippocampal neurons. An altered balance in the antioxidant enzyme systems following KA could mediate the neurodegenerative process.

836.22

NITRIC OXIDE SYNTHASE-CONTAINING CELLS IN THE RAT HIPPOCAMPUS ARE RESISTANT TO TRIMETHYLTIN. H. Kanai*, M. Ikeda, M. Akaike, S. Tsutsumi, Y. Takahashi, M. Sadamatsu, A. Masui, and N. Kato, Dept. of Psychiatry, Shiga Univ. of Med. Sci., Otsu 520-21, Dept. of Physiol. Chem., Tokyo M & D Univ. Graduate School, Tokyo 113, and Drug Develop. Res. Labs., Hoechst Japan Ltd., Kawagoe 350-11, Japan.

Nitric oxide (NO) is implicated both in the physiology and the pathology in the hippocampus. We studied change in NO activity in the hippocampus of rats treated with trimethyltin hydroxide (TMT; 9mg/kg, p. o.), by means of morphological NADPH-diaphorase staining and biochemical enzymatic determination of NO-synthase. TMT was known to induce selective loss of CA3/4 pyramidal cells in the hippocampus.

The rats were sacrificed 5 and 16 days after TMT treatment . TMT exposure caused the typical behavioral changes of TMT intoxication such as hyperactivity, aggression, and tail-mutilation, as well as the hippocampal damage as reported previously. The interneurons scattered in the hilus and CA3/4 were found strongly positive in NADPH-diaphorase staining. The CA1 pyramidal cells, which were reported to contain endothelial NO synthase, also had weak positive staining. Both positive cells were not affected by TMT treatment. NO synthase activity was measured by the conversion of [3H] arginine to [3H] citrulline. [3H] citrulline was separated from [3H] arginine by high-performance liquid chromatography and counted by liquid scintillation. There was no significant change in NO synthase activity in the hippocampus between control and TMT-treated rats. Despite these behavioral and morphological changes, NADPH diaphorase staining remained unchanged, which was further substantiated by no change in NO synthase activity. These results suggest the resistance of NO synthase-containing neurons to TMT.

836.23

EXPRESSION OF NOS ISOFORMS IN THE DEVELOPING AND DEGENERATED RAT BRAIN: MORPHOLOGICAL; RT-PCR; WESTERN BLOT AND MICRODIALYSIS STUDY

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Nitric oxide synthase (NOS) in the brain is present in different isoforms, which possess a histochemically detectable NADPH-diaphorase (NADPH-d) activity. To test when these isoforms first appear during ontogenesis, mRNA and protein levels of brain (b), endothelial (e) and inducible (i) NOS in tissue samples from pre- and postnatal rat brains were determined and compared with the ontogenetic pattern of NADPH-d activity obtained by histochemistry. Starting with the embryonic day (ED) 10 the mRNA-levels of all isoforms showed no or only minor changes until adult state in all brain regions except i-NOS, which temporally declined by 17th ED. In contrast to the permanent expression of the specific RNAs, but in line with the ontogenetic pattern of the NADPH-d at the light microscope, detectable amounts of b- and i-NOS in the Western blots appeared first at ED 15 and increased considerably until adult state. Endothelial-NOS protein, however, was expressed during all developmental stages and increased moderatly until adult state. Ultrastructurally, NADPH-d labeling was seen in the brain pallium of 13-day-old embryos. The reaction product was deposited on membranes of the nuclear envelope. Cells of ED 10 were, as a rule, free of formazan.

Exposure to quinolinic acid led to a massive loss of neuronal cells in the striatum. Parallel to this depletion a striking proliferation of reactive microglia/macrophages and astroglia was seen. Most of IB_4 positive macrophages showed NADPH-d reaction product deposited on membranes, whereas the population of highly phagocytotic cells displayed cytosolic staining, which seems to be the inducible isoforms. Parallel microdialysis studies showed a significant increase in citrulline 7 days after intoxication by which the induction effect on NOS production is further substantiated.

NEURO-ONCOLOGY: TUMOR BIOLOGY

837.1

BROMODEOXYURIDINE UPTAKE IN RG-2 RAT BRAIN TUMORS: LIMITATIONS OF A PROLIFERATION MARKER.

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Bromodeoxyuridine (BrDU) is used as a proliferation marker and radiosensitizer in brain and other tumors. We looked at incorporation of ^{14}C-BrDU into a nonextractable tissue fraction in normal and neoplastic tissue. RG-2 gliomas were transplanted into 12 male Fischer-344 rats. Each animal received a 50 μCi bolus of ^{14}C-BrDU in a femoral vein; multiple blood samples were collected in 10, 30, and 60 min experiments. Total ^{14}C activity and BrDU concentration was measured in plasma. The amount of labeled BrDU in 20 μm thick sections was measured with quantitative autoradiography, before and after washing with methanol. We calculated a plasma half-life of BrDU, an apparent blood-to-tissue transport value (K_{1app}), and an incorporation quotient (Q). Plasma half-life was < 10 min. The K_{1app} decreased rapidly in RG-2 tumor, from 15 μl/g/min at 10 min to 5 μl/g/min at 60 min. In cortex, K_{1app} peaked at 30 min at 3 μl/g/min. Q in RG-2 tumor was constant from 10 to 30 min, at 3 μl/g/min, and peaked at 30 min in cortex at 3 μl/g/min. BrDU is rapidly metabolized ; its availability is limited by capillary permeability. In rapidly proliferating tissue with low permeability, BrDU uptake is likely to underestimate the true rate of proliferation.

837.2

EXPRESSION OF DIAZEPAM BINDING INHIBITOR AND MITOCHONDRIAL BENZODIAZEPINE RECEPTOR IN HUMAN ASTROCYTOMAS: RELATIONSHIP TO CELL PROLIFERATION.

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A polypeptide capable of displacing benzodiazepine binding from both central- and peripheral-type receptors has been purified from the brain and liver of different species and has been designated as diazepam binding inhibitor (DBI). The expression of diazepam binding inhibitor and mitochondrial benzodiazepine receptor (MBR) were studied in human astrocytic tumors using immunocytochemistry and *in situ* hybridization. Both MBR and DBI were prominently expressed in neoplastic cells, whereas low or undetectable levels were present in normal brain. Immunocytochemical double-staining demonstrated that MBR and DBI were present in the same cells, suggesting that DBI may act in an autocrine manner in these cells. Analysis of 86 cases showed that MBR expression was statistically significantly associated with tumor malignancy grade (p=0.004) and proliferative index as determined by MIB-1 immunocytochemistry (p=0.004). Patients having tumors with high number of MBR immunoreactive cells had shorter life expectancy than patients whose tumors showed lower MBR expression (p=0.024). These results suggest that MBR might be useful in evaluating malignancy in brain tumors.

837.3

VASOACTIVE INTESTINAL PEPTIDE IMMUNOREACTIVITY AND mRNA ARE PRESENT IN A HUMAN ASTROCYTOMA CELL LINE.

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Vasoactive intestinal peptide (VIP) is a potent neuroendocrine mediator with a wide range of biological activities. In addition to its role as a neurotransmitter, VIP is a potent survival factor for cultured cortical neurons, and has been shown to stimulate mitosis in primary astrocytes and cultured mouse embryos. VIP immunoreactivity is present in many human tumors, and VIP receptors have been found in colonic adenocarcinomas, breast cancers, melanomas, neuroblastomas, and pancreatic carcinomas. In the CNS, VIP is present in neurons, but has not been found in primary astrocytes. Given the vigorous growth factor effects of VIP, and the prevalence of VIP in other tumor cells, we investigated the presence of VIP in a human astrocytoma cell lines. The grade IV human astrocytoma cell line U373 was grown in minimal essential medium with 10% fetal bovine serum. Conditioned medium was obtained by incubating for 6 hours in serum-free media. Total RNA was extracted from cell pellets, and reverse transcription- polymerase chain reaction (PCR) was performed using primers highly specific for VIP mRNA. PCR products were obtained from these samples within 30 cycles. The identitiy of PCR products was verified using nested primers. In addition, VIP peptide immunoreactivity in both U373 cell pellets and conditioned media was investigated using radioimmunoassay. VIP immunoreactivity in cell pellets was detected and correlated with density, increasing from 162.5 pg/flask with 30-40% confluence, to a mean of 812.7 pg/flask with 100% confluence. In the media, VIP immunoreactivity was also found at a level of 15 pg/ml regardless of confluence. These data indicate that a human astrocytoma cell line has both mRNA and immunoreactivity for VIP.

837.4

REVERSE-TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR) ANALYSIS OF SOMATOSTATIN RECEPTOR (SST) EXPRESSION IN NEUROBLASTOMA.

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Somatostatin receptor (SST) expression indicates good prognosis in neuroblastoma†. Five SST genes (SST1-5) have been cloned. This study investigated the expression of SST1-5, somatostatin peptide (SMS), and a control gene (c-abl) in neuroblastoma. RT-PCR was used to analyze SST1-5, SMS, and c-abl gene expression in total RNA isolated from 13 neuroblastoma tumor samples, 3 neuroblastoma cell lines, and 3 control tissues: pituitary, brain, and adrenal. The tissues were provided by the Cooperative Human Tissue Network at Children's Hospital and The Ohio State University Hospitals. Tissues were kept at -80°C with or without cryoprotectant. RNA was demonstrated to be free of genomic DNA (gDNA) using primers for the c-abl gene designed to span an intron. The c-abl cDNA product but no gDNA product was demonstrated in 13 neuroblastoma samples, 3 cell lines and 3 control tissues. Eight of 13 neuroblastoma tumors, 3/3 control tissues and 2/3 neuroblastoma cell lines demonstrated SST2 expression. No neuroblastoma tumors or cell lines tested to date have expressed SST1, SST3, SST4, or SST5. Pituitary expressed SST1, SST3, and SST4 but not SST5. Brain expressed SST1, SST4, and SST5. The cDNA product for SMS was demonstrated in 12/13 neuroblastoma samples and in none of the cell line samples. The current study suggests that RT-PCR SST expression analysis of total RNA extracted from small tissue samples, eg. tumor biopsies, provides a powerful assay to use 1) in determining prognosis in cancer and 2) as a basis for choosing to use somatostatin analog therapy in cancer.

†O'Dorisio, M.S., F. Chen, T. M. O'Dorisio, D. Wray, and S. Qualman. 1994. Characterization of somatostatin receptors on human neuroblastoma tumors. *Cell Growth & Differentiation* 5:1-8.

837.5

EFFECT OF INTERFERON AND NERVE GROWTH FACTOR ON GLIOMA GROWTH CORRELATES WITH ALTERATION OF CELL SIZE. M. Wiranowska[1], S.Saporta[2], J.Moore[2], S.Phuphanich[1] and M.F. Nolan[2]*. Departments of Neurology[1] and Anatomy[2], College of Medicine, University of South Florida, Tampa, FL., 33612.

The effects of interferon (IFN), nerve growth factor (NGF) and IFN+NGF on human astrocytoma U-373, human melanoma A-375 and mouse glioma G-26 cell growth, size and differentiation evaluated here. Cells were incubated for 3 days with NGF at 0.1-10 µg/ml, 5-5x10^4 IU/ml of human recombinant IFNα-2b or 8x10-8x10^4 IU/ml of mouse IFNα/ß, or with a combination of NGF and IFN. Cell growth was evaluated by incorporation of 3-H thymidine and cell cycle by analysis with flow cytometry. Morphology and cell size were evaluated microscopically and with a Coulter Multisizer. It was found that homologous IFNs inhibited growth of all evaluated cells in a dose-dependent manner, while NGF inhibited only glioma cells showing synergy with IFN. Following NGF/IFN treatment, the number of smaller glioma cells increased while the number of large cells decreased. Unique "spider-like" cells with long processes were seen microscopically following NGF/IFN treatment. Cell differentiation was evaluated by expression of glial fibrillary acidic protein (GFAP) by Western immunoblotting. The GFAP levels of NGF/IFN treated mouse glioma G-26 cells increased, compared to the controls, which have a low initial expression of GFAP. Human U-373, which expresses high initial GFAP, demonstrated no detectable change in GFAP levels. It is postulated that the synergistic inhibitory activity of NGF/IFN on glioma proliferation correlates with the increased number of small glioma cells, which may be an indication of glioma cell differentiation into a non-proliferative phenotype.

837.6

ALTERATIONS IN CYCLIN B1 EXPRESSION AND CELL CYCLE KINETICS IN HUMAN GLIOMA AND MEDULLOBLASTOMA FOLLOWING RADIATION AND CHEMOTHERAPY. AJ Janss*, A Maity, GD Kao, RJ Muschel, WG McKenna, PC Phillips. Div. of Neurology, Children's Hospital of Philadelphia and Depts. of Radiation Oncology and Pathology and Laboratory Medicine, Univ. of Pennsylvania Medical Center. Philadelphia , PA.

Cell cycle check points mediate responses to DNA-injury in proliferating cells. Cyclin B1 has been implicated in the regulation of cellular commitment to mitosis (G2/M transition) in eukaryotic cells yet little is know about its role in treatment-induced G2 delay in brain tumors. Gliomas are common, treatment-resistant brain tumors in adults, while medulloblastomas are common, relatively treatment-sensitive brain tumors in children. To study cyclin B1 in these brain tumors we treated synchronized U251 glioma cells and DAOY medulloblastoma cells during S phase with 6Gy radiation (RT) or 100nM camptothecin (CPT; 1 hr exposure). Cell cycle kinetics were evaluated using flow cytometry, cytotoxicity using clonogenic assays, nuclear integrity and membrane integrity, and expression of cyclin B1 mRNA and protein using northern and western blot analysis, respectively. Glioma cells exposed to RT or CPT were delayed in G2 and had decreased cyclin B1 protein and mRNA. Medulloblastoma cells exposed to CPT also exhibited G2 delay and decreased cyclin B1 mRNA. In contrast, irradiated medulloblastoma cells had little G2 delay and a less marked decrease in cyclin B1 mRNA. CPT produced nuclear fragmentation and loss of membrane integrity in medulloblastoma cells within 48 hr or treatment, while RT did not. We conclude that cyclin B1 is involved in production of treatment-induced G2 delay in both human glioma and medulloblastoma cells. We speculate that lack of cyclin B1 protein alterations and G2 delay in medulloblastoma cells after RT may be one mechanism explaining the relative sensitivity of medulloblastomas to RT. Check points in cell cycle progression may represent important targets for anti-tumor therapy as they may modulate cellular sensitivity to DNA-damaging agents.

837.7

mdm2 GENE INDUCES THE EXPRESSION OF mdr1GENE AND P-GLYCOPROTEIN IN A HUMAN GLIOBLASTOMA CELL LINE S. Kondo*[1], G. H. Barnett[1], T. Morimura[2] and J. Takeuchi[2]. [1]Department of Neurosurgery/S80, Brain Tumor Center/Cancer Center, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Clevaland, OH 44195, U.S.A.; [2]Department of Neurosurgery, National Utano Hospital, Ukyo-ku, Kyoto 616, Japan.

The overexpression of multidrug resistance (mdr1) gene and its product, P-glycoprotein (Pgp), is thought to limit the successful chemotherapy of human tumors. Recent studies demonstrate that mutations of the tumor-suppressor gene p53 may enhance the expression of mdr1 gene and Pgp. However, as there are some human tumors that overexpress both mdr1 gene and Pgp but reveal no mutations of p53, the mechanism by which mdr1 gene and Pgp are overexpressed in human tumors is not yet clear. In this report, we show that mdm2 (murine double minute 2) gene induces the expression of mdr1 gene and Pgp in human glioblastoma U87-MG cells expressing neither MDM2 nor Pgp. Furthermore, mdm2 confers the resistance of U87-MG cells to apoptotic cell death induced by etoposide (VP-16) and adriamycin. These findings suggest the possibility that mdm2 may play an important role in the development of MDR phenotype in human tumors.

837.8

NEUROBEHAVIORAL TESTING AS A PREDICTOR OF TUMOR VOLUME WITHIN THE RODENT CAUDATE NUCLEUS. P.D. Sawin, R.M. Roach, V.C. Traynelis*. The University of Iowa College of Medicine, Iowa City, IA 52242

Introduction: Brain injury resulting from intracranial tumor growth is dynamic and progressive. The purpose of this study was to correlate neurobehavioral findings with tumor volume in a rodent model of intracranial glioma.

Methods: 24 adult male Fischer 344 rats were assigned randomly to one of four groups. In Groups 1-3, a 36B10 glioma (5000 cells/inoculum) was implanted into the dominant caudate nucleus of each animal. Group 4 animals served as sham-operated controls. Animals in Groups 1, 2, and 3 were sacrificed at 8, 12, and 16 days post-implantation, respectively. Tumor size was quantified by computerized volumetric analysis. All animals underwent a battery of neurobehavioral tests preoperatively and immediately prior to sacrifice.

Results: All implanted animals developed viable tumors. Mean tumor volume increased over time (1.67±0.80 mm^3 @ 8 days, 3.54±1.98 mm^3 @ 12 days, 9.98±4.29 mm^3 @ 16 days). No significant difference in Bederson motor performance was noted between groups. Dual simultaneous stimulation (tape test) revealed greater contact and removal asymmetry with greater tumor size. Parallel bar testing demonstrated deterioration in performance with increasing tumor size ($p=0.005$). Native rotational behavior was reduced in tumor-bearing animals ($p<0.001$). Apomorphine enhanced turning behavior in these animals ($p=0.001$), and increased the magnitude of contralateral rotation bias ($p=0.02$). Sham animals did not demonstrate increased rotation with apomorphine.

Conclusions: Neurobehavioral changes induced by an enlarging lesion within the rodent caudate are subtle. Increasing tumor volume is associated with progressive tape test asymmetry, decline in parallel bar performance, and altered rotational activity. These neurobehavioral findings facilitate estimation of tumor volume *in vivo*, enhancing the utility of rodent brain tumor models.

NEURO-ONCOLOGY: TREATMENT

838.1

GENE THERAPY FOR BRAIN TUMORS BY AUTOLOGOUS VACCINATION WITH ENGINEERED GM-CSF SECRETING TUMOR CELLS. J.S. Yu, J.A. Burwick, G. Dranoff, E.A. Chiocca* and X.O. Breakefield Neurosurgery Service and Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, MA 02129 and Dana-Farber Cancer Insitute, Boston, MA 02125

We have developed an ex vivo gene therapy paradigm for the treatment of brain tumors using granulocyte-macrophage colony-stimulating factor (GM-CSF). The murine B16 melanoma and the GL 261 glioma lines were infected with MFG recombinant retrovirus containing the mouse GM-CSF cDNA. The tumor lines were confirmed to secrete GM-CSF by ELISA. Subcutaneous vaccination of syngeneic mice with irradiated GM-CSF-secreting B16 melanoma cells completely protected animals from subsequent intracranial B16 tumor inoculation. Histologic evaluation revealed the presence of neutrophils, eosinophils and lymphocytes in the intracerebral inoculation site. In contrast, animals vaccinated with irradiated B16 cells or not vaccinated succumbed to intracranial tumor within 3 weeks after inoculation. Treatment of established intracranial B16 melanoma tumors with subcutaneous injection of irradiated GM-CSF secreting B16 cells increased median survival as compared to injection of irradiated B16 cells or no treatment. In addition, treatment of intracerebral glioma with subcutaneous vaccination with irradiated, GM-CSF-secreting autologous cells also increased survival compared to controls. Histologic examination revealed a dramatic perivascular lymphocytic infiltrate beginning 3 days after treatment with GM-CSF transduced cells which was not found in controls.

838.2

EXTENDED TRANSFER OF HSV-TK GENE INTO DISSEMINATED 9L BRAIN TUMORS BY INTRAARTERIAL AND INTRAVENTRICULAR DELIVERY OF A HERPES VECTOR. C.M. Kramm, N.G. Rainov, M. Chase, M. Sena-Esteves, E.A. Chiocca, X.O. Breakefield*. Molecular Neurogenetics Unit, Massachusetts General Hospital, Boston, MA.

The aim of the present study was to improve delivery of therapeutic herpes simplex virus type 1 thymidine kinase (HSV-TK) gene into disseminated brain tumor masses by intraarterial and intraventricular injection of vectors. The herpes vector hrR3 was used because highest gene transfer efficiency was achieved after intratumoral application in our laboratory with this vector, as compared to retrovirus or adenovirus vectors. hrR3 bears the HSV-TK and the E. coli *lacZ* reporter gene and has a deletion in the ribonucleotide reductase gene, which allows selective virus replication in dividing cells. Two different models of disseminated 9L brain tumors were established 5-7 days before intraarterial or intraventricular injections of hrR3. For intraarterial application bradykinin was injected prior to the vector to disrupt selectively the blood-tumor-barrier. Transfer efficiency of the HSV-TK and *lacZ* genes was investigated in multiple tumor foci throughout both hemispheres. Intraventricular delivery of hrR3 was achieved via stereotactical injections into the cisterna magna, and distribution of the HSV-TK and *lacZ* genes was evaluated in a model with a right frontal lobe tumor and extensive leptomeningeal tumor spread throughout the CSF pathways. HSV-TK and *lacZ* gene delivery was assessed in tumor masses and in normal brain at different time points by immunohistochemical and histochemical detection of the gene products. With intraarterial application of hrR3 following selective disruption of the blood-tumor barrier by bradykinin, transfer of the HSV-TK and *lacZ* genes was found almost exclusively in the periphery of all disseminated tumor foci. Normal brain was not affected, and no toxicity was observed. Intraventricular delivery of the vector resulted in expression of the HSV-TK and *lacZ* genes in the intracerebral and the leptomeningeal tumor masses. Some toxicity was observed with intensive leptomeningeal inflammation and temporary infection of periventricular areas of the brain. Intratumoral HSV-TK and *lacZ* expression was strongest within the first 2 days after intraarterial or intrathecal herpes injection, but was still detectable up to at least 16 days after virus application, consistent with vector replication in the tumors. Both application strategies provide a marked extension of TK gene transfer efficiency, as compared to intratumoral delivery. [Study supported by NINDS grant NS24279. CMK is a fellow of the Deutsche Krebshilfe. NGR is a Wilhelm-Toennis-scholar.]

838.3

REPLICATION OF HERPES SIMPLEX VIRUS-1 GAMMA 34.5 DELETION MUTANT IN MURINE BRAIN TUMORS.

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Herpes simplex virus γ- 34.5 deficient viruses are being considered as candidates for use as cancer therapy vectors. Studies have shown that the neurovirulence factor γ-34.5 appears necessary for replication in the central nervous system. Previously we have shown the efficacy of HSV type I (HSV-1) strain 1716, which has a deletion in the γ34.5 neurovirulence gene, to induce selective lysis of human tumor cells in the nude mouse brain. We are expanding these studies to immunocompetent mouse tumor models to determine the effects of a functional immune system on viral therapy. Preliminary immunohistochemical and in situ hybridization studies suggest that tumor lysis does occur in the presence of an inflammatory response. We are currently determining the role of this response in modulating the efficacy in viral therapy.

838.4

IMMUNIZATION AND GANCICLOVIR TREATMENT DELAY THE FORMATION OF HSV-TK EXPRESSING BRAIN TUMORS IN RATS THROUGH IMMUNOLOGIC SUPPRESSION.

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Immunity to tumors develops following ganciclovir (GCV) treatment of brain tumors expressing the Herpes Simplex Virus-thymidine kinase (HSV-TK) gene. To study if HSV-TK by itself might contribute to the anti-tumor immunogenicity, the growth of non-immunogenic D74 tumors, wild type (D74-w.t.) and TK-modified D74 (D74-TK) brain tumor cells, were studied in naive rats and rats previously treated and cured of 9L tumors by HSV-TK and GCV treatments. Formation of the D74-TK tumors were significant reduced in both naive and pretreated rats, even though the *in vitro* growth rate of these tumors was similar. Analysis of immune cells infiltrating the tumors revealed a significant increase in the CD4 positive cells in TK-modified tumor cells in the previously treated rats. Furthermore the regression of the D74-TK tumors was greater in the previously treated rats. These findings suggest that even in non immunogenic tumors, the expression of HSV-TK slows the tumor formation, that can be associated with the immune response. This immunity could be exploited by vaccination strategies to increase tumor regression seen with HSV-TK and GCV treatments.

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838.5

ADENOVIRUS-MEDIATED GENE TRANSFER INTO EXPERIMENTAL RAT GLIOMAS.

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Adenovirus-mediated transfer of cytokine genes is a novel technique which may have therapeutic value in the treatment of central nervous sytem neoplasms. It has not been established however, that viral recombinants can transduce different brain tumors with similar efficiency. Also, we wanted to investigate whether direct viral-mediated transfer of cytokines into a pre-established tumor could elicit signs of tumor rejection due to increased activation of the host's immune system. In order to address these issues, we transduced five different rat glioma cell lines with two different adenoviral recombinants, one carrying the βgal reporter gene (AdCMVβgal), and the other containing the cDNA for human interleukin-2 (AdCMVIL2). Even at a multiplicity of infection of 1000 we observed a strong difference of infectability between the different glioma cell lines as indicated by X-gal histochemistry and by the level of Il-2 released into the culture media. This variance was consistent for both adenoviral recombinants. The degree of infection efficiency of the gliomas from highest to lowest was D74 > F98 > C6 > 9L > T9. X-gal histochemistry revealed that approximately 95% of D74 cells were transduced as compared to approximately 1% of T9 cells. In addition, we are testing for the ability of AdCMVβgal to transduce *in situ* gliomas. Adenoviral-mediated cytokine transfer into established gliomas will be discussed.

838.6

A NOVEL GENE THERAPY APPROACH TO PITUITARY ADENOMAS.

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Although dopamine (DA) receptor agonists play a role in managing prolactinomas, there are significant side-effects. We are developing a gene therapy approach to prolactinomas which would eliminate the need for such drugs and which has applications to other adenomas. We have constructed viral vectors containing genes encoding DA biosynthetic enzymes, including tyrosine hydroxylase (TH) and amino acid decarboxylase (AADC). By introducing such vectors into a prolactinoma, DA levels might increase locally, inhibit prolactin secretion and shrink the tumor.

Using an Adenovirus vector with a TH gene (AdCMVth), transfection of human prolactinoma cultures resulted in transgene expression, as monitored by immunocytochemistry. Increases in dopamine release were observed in cultures treated with AdCMVth, compared to controls. Significant and sustained reductions of secreted prolactin were also observed in AdCMVth treated cultures, compared to controls.. Similar results were obtained using an Adeno-associated virus vector which contains transgenes encoding both human TH and AADC.

Viral vectors might be used for gene therapy in other pituitary adenomas by introducing transgenes encoding proteins which modulate hormone secretion and tumor growth patterns. Unlike strategies based on introducing cytotoxic genes into tumors, our approach doesn't require that virtually all tumor cells are transfected to achieve these effects.

838.7

INHIBITION OF GLIOMA GROWTH IN RATS TREATED WITH INTERLEUKIN 2 GENE-MODIFIED ENDOTHELIAL CELLS.

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Interleukin-2 (IL-2) is known to be secreted by T helper cells and to stimulate cytotoxic T cells and natural killer cells, affecting antitumor responses. The antitumor effects of IL-2 were examined in rats implanted with 9L glioma cells. As a delivery vehicle of IL-2, rat brain endothelial cells immortalized with adenovirus E1A gene and modified to express the *lac Z* (RBEZ) were used. RBEZ cells have been shown to survive for greater than 1 month after implanting to brain tumors. RBEZ-IL2 cells were obtained by transfection with pBCMG-hygro-IL2, a BPV expression vector containing murine IL-2 cDNA under the transcription control of a cytomegalovirus promoter. 9L cells ($1X10^5$) with either RBEZ-hygro (control) or RBEZ-IL2 cells ($2X10^6$) were injected subcutaneously into flanks of rats and tumor volumes sequentially measured. RBEZ-IL2 treatment inhibited tumor growth (at 27 days, 13635 ± 2883 vs 170 ± 144 mm^3, n=10, p=0.001). 9L gliomas were also injected stereotactically into left cerebral hemispheres concurrent with either RBEZ-hygro or RBEZ-IL2 cells. After 14 days, rats were sacrificed and cross-section areas and tumor volumes were determined. Endothelial-based IL-2 delivery prevented intracranial glioma formation as estimated by cross-section area (35 ± 3 vs 6 ± 5 mm^2, n=3, p=0.006) and tumor volume (214 ± 32 vs 26 ± 25 mm^3, n=3, p=0.005). RT-PCR for IL-2 was performed to document IL-2 gene expression in vivo. IL-2 specific PCR products were detected at 5 days post-implantation in hemispheres receiving RBEZ-IL2 cells but not in contralateral hemispheres. These findings establish that genetically modified endothelial cells can be stably engrafted to growing gliomas and effectively deliver antitumor agents.

838.8

ANTIPROLIFERATIVE EFFECT OF C-MYC ANTISENSE PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDES IN RAT GLIAL TUMOR CELLS.

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Antisense oligodeoxynucleotides (ODNs) offer the potential to block the expression of specific genes within cells. c-myc is an immediate early response gene induced by various mitogens, suggesting that its protein product may play a role in numerous signal transduction pathways, including those modulating cell division. Rat-specific phosphorothioate 15-mer ODNs were synthesized by the Nucleic Acids Synthesis and Analysis Laboratory of the Massey Cancer Center. The antisense c-myc sequence used was as follows: 5'-CACGTTGAGGGGCAT-3'. Three concentrations of ODNs (1uM, 3uM, 10uM) were added to the media of cultures of RT-2 cells (a rat glioblastoma cell line), and cell growth was assessed by MTT assay one to four days after adding the ODNs. Compared to cultures containing standard media, antisense oligodeoxynucleotide was found to significantly inhibit the growth of glioma cells, while sense and scrambled sequence ODNs did not significantly affect cell growth at the concentrations tested. Western blot analysis showed that expression of immunoreactive c-myc protein was also reduced in the antisense ODN-treated cells (and not in sense-, scrambled- or control-treated cells). Flow cytometry and immunocytochemistry studies are ongoing. These results suggest that c-myc plays a critical role in glioma cell proliferation, and support a potential role for antisense strategies designed to inhibit c-myc expression in the treatment of malignant gliomas in clinical settings.

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838.9

Suppression of Human U87 Glioblastoma Tumor Growth in the Flank of Nude Mice with Antisense Oligonucleotides to the *c-myb* Oncogene. W. C. Low*, C. You, E. P. Flores, L. Chiang, J. A. Conrad, X. Q. Liu, D.Y.K. Wen, and W. A. Hall. *Departments of Neurosurgery and Physiology, and Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455*

c-myb is a proto-oncogene which encodes a nuclear protein involved in the regulation of the cell cycle. This oncogene is typically over expressed in various types of cancer. Previous studies in our laboratories demonstrated that antisense oligonucleotides to the *c-myb* oncogene can suppress the proliferation of human medulloblastoma and glioblastoma cells grown *in vitro*. In the present study, we have examined the effects of antisense administration *in vivo* using nude mice as tumor transplant recipients. Human glioblastoma of the U87 cell line were injected into the flank of nude mice (5×10^6 cells). Ten days after the injection of the tumor cells, animals received injections of either *c-myb* antisense or nonsense (100 μM), or Hank's balanced salt solution (HBSS) in 50 μl volumes directly into the tumor. Antisense oligos were designed for codons 182-188 of the *c-myb* oncogene. Antisense injections were given at days 10, 12, 14, and 16 after the transplantation of the tumor cells. Tumor volume (mm^3) was measured daily for each animal over a period of 17 days. Animals given injections of HBSS exhibited tumors at day 17 which significantly increased in size to 217% ($p < 0.05$) of the volume prior to HBSS administration. Animals given nonsense oligonucleotides also exhibited tumors which significantly increased in size by 210% ($p < 0.05$). In contrast, animals receiving *c-myb* antisense exhibited no significant increase in tumor size with tumors only 128% of their pre-antisense treatment size. These results suggest that the administration of antisense to codons 182-188 of the *c-myb* oncogene can suppress U87 tumor growth *in vivo*, and may thus be an effective form of therapy in treating human brain tumors. *(Supported by a grant from the American Cancer Society).*

838.10

LAZAROIDS INHIBIT PROLIFERATION OF CULTURED HUMAN BRAIN TUMOR CELL LINES. P. Arora, Y.S. Lee, T.C. Origitano and R.D. Wurster*. Neuroscience Program, Depts. of Physiology and Neurological Surgery, Loyola Medical Center, Maywood IL 60153.

Lazaroids (or 21-aminosteroids) are potent lipid peroxidation inhibitors having antioxidant properties (i.e. scavengers of reactive active species, ROS) similar to glucocorticoids and vitamin E. ROS, at low levels, are shown to activate signal transduction pathways (Ca^{2+}, PKC and arachidonic acid) and induce expressions of certain proto-oncogenes (c-fos, c-jun, c-myc) that are involved in cell proliferation. In this study cultured human brain astrocytoma (U-373 MG) and neuroblastoma (SK-N-MC) cell lines were used to determine if two representative lazaroid compounds (U-74389G and U-83836E) could affect tumor cell proliferation. Alpha-tocopherol (or vitamin E, a potent antioxidant) and steroids (methylprednisolone and dexamethasone) were also tested under similar experimental conditions. Cell viability was assessed by counting viable cells in a hemocytometer using the trypan blue exclusion method. Results obtained show that both lazaroids effectively inhibit proliferation of U-373 MG and SK-N-MC in a dose-dependent manner. The steroids and alpha-tocopherol were also antiproliferative but at higher doses. In addition to their antioxidant effects, the antiproliferative effects of lazaroids may also be attributed to their ability to chelate iron and decrease membrane fluidity. In summary, lazaroids have significant growth-inhibitory properties that may have potential clinical use in the treatment of certain brain tumors, especially where ROS are known to be involved in tumor promotion. They would be clinically advantageous as their actions are devoid of the usual side effects of steroid therapy and are more potent than alpha-tocopherol.

838.11

INHIBITION OF GLIOMA GROWTH WITH PROTEIN TYROSINE KINASE INHIBITOR: METHYL 2,5-DIHYDROXYCINNAMATE. S Yazdani, CG Caday*, X Alvarez and A Nanda. Division of Neurosurgery, Department of Surgery and Biomedical Research Institute, Louisiana State University Medical Center, Shreveport, LA 71130.

Many protein tyrosine kinases (including growth factor receptors and oncogenes) are overexpressed in human gliomas. Thus, selective inhibition of specific protein tyrosine kinases (PTK) is a potential therapeutic approach to inhibit glioma proliferation. Erbstatin selectively inhibits epidermal growth factor receptor (EGFR) tyrosine kinase activity, but it is unstable. Repeated treatment with methyl 2,5-dihydroxycinnamate (MDHC), a more stable member of the erbstatin family of PTK inhibitors, inhibited glioma growth in culture with an IC_{50} of 2-3 μM. More than 90% of cell growth was inhibited following repeated treatment with 5 μM MDHC. In contrast, glioma cultures that were treated once with MDHC (at concentrations as high as 20 μM) were able to overcome the MDHC inhibition and regrow. The IC_{50} of MDHC showed that it was a more potent inhibitor than the structurally homologous tyrphostins which inhibited gliomas with an IC_{50} of 20-40 μM [Miyaji *et al.* 1994 J Neurosurg 81: 411-419]. MDHC-treated glioma cells did not appear to die by necrosis. MDHC-treated cells showed retraction of glial processes, cell shrinkage and enhanced DAPI staining. The potential use of members of the erbstatin family for glioma therapy requires development of more stable analogues with more specific tyrosine kinase activity.

838.12

Induction of programmed cell death by 1,25-dihydroxyvitamin D3 in C6.9 rat glioma: A role for 1,25-(OH)2D3 in the control of brain tissular homeostasis? D. Wion, C. Baudet, P. Naveilhan, G. Chevalier, P. Brachet* INSERM U 298, CHU Angers, F-49033 Angers, France.

1,25-dihydroxyvitamin D3 (1,25-(OH)2D3) is a seco-steroid hormone which exerts its principal biological activities through specific nuclear receptors (VDR). Accumulating evidence, including the presence of VDR in several brain areas, the synthesis of 1,25-(OH)2D3 by activated microglial cells and the regulation of neurotrophins expression by 1,25(OH)2D3 in astrocytes, suggests that the brain is a target for this hormone.

We have previously reported that 1,25-dihydroxyvitamin D3 exerts a toxic effect in rat C6 and human GHD glioma cell lines (Naveilhan et al. J. Neurosci. Res. (1994), 37, 271). In view of the well known heterogeneity of C6 cells, a C6 subclone called C6.9, isolated by the method of limiting dilution, was used to investigate the underlying mechanisms by which 1,25-(OH)2D3 acts on glioma cells. When C6.9 cells cultured in a serum-free medium were treated during 24 hours with 10^{-7}M 1,25-(OH)2D3, cell death occured 5-7 days later. An increased expression of c-myc, gadd 45, and p53 genes is observed at day 3. Apopototic DNA fragmentaion is also observed on day 6. However, nuclear fragmentation, which is the morphological criteria for apoptosis, was not observed. Taken together these data suggest that 1,25-(OH)2D3 induces a programmed cell death in C6.9 cells which shares the biochemical but not the morphological features of apoptosis. They also offer new therapeutic perspectives in the treatment of gliomas. Furthermore, they raise the possibility that the synthesis of 1,25-(OH)2D3 by activated microglial cells could limit the appearance of brain tumors and then add another clue on the possible roles of 1,25-(OH)2D3 in the nervous system.

838.13

OLIGOSACCHARIDE INHIBITION OF GLIOMA GROWTH M. Nieto-Sampedro, C. Bailón, A. Fernández-Mayoralas, M. Martín-Lomas, B. Mellström, and J.R. Naranjo*, Cajal Institute and Institute for Organic Chemistry, CSIC, Madrid, Spain.

α–D-GalNAc-(1→3)-β-D-Gal-(1→4)-[α–L-Fuc-(1→3)]-β-D-GlcMe (TS4; Me, methyl), a synthetic analogue of an antimitotic present in brain[1], was cytostatic for cultured primary rat astrocytes and rat C6 glioma cells at μM concentrations[2], but showed no cytotoxicity at concentrations 100-fold above the ID50. The growth of tumors formed after C6 transplantation in brain was, as expected, inhibited by continuous infusion of TS4 (10 μg/hour). In addition, treated tumors appeared necrotic, suggesting that TS4 may have activated cell-mediated destruction mechanisms. Natural killer (NK) cell activation, requires binding of an oligosaccharide ligand to the receptor lectin NKR-P1; these ligands have a structure[3] very similar to TS4. On the other hand, TS4-induced conexin 43 overexpression, could enhance C6 immunogenicity and cause cytotoxic lymphocyte activation. TS4 and sialyl-Lewis are closely related to blood groups and selectin ligands. Their antimitotic properties[2] suggests a role for blood group carbohydrates and may account for contact inhibition of cell proliferation. This work introduces a new class of antimitotics and potential antimetastatics. Supported by grants SAF 0212-92 from the CICYT, Spain, and by Boehringer Ingelheim España, S.A..

1. Nieto-Sampedro (1988) *Science* 240, 1786. 2. Coteron et al. (1995) *J. Org. Chem.*, in press. 3. Bezouska et al. (1994) *Nature* 372, 150.

838.14

BRAIN GLIOMA TREATMENT BY MONOCLONAL ANTIBODIES TO HUMAN CHORIONIC GONADOTROPIN. M.L. Leavitt*, E.A. Acevedo, H.F. Acevedo, A. Krichevsky, R. Gorewit, and J.C. Maroon. Allegheny-Singer Research Inst., Medical College of Pennsylvania and Hahnemann Univ., Pittsburgh, PA 15212 and InVitro Technologies, Inc., Pittsburgh, PA 15238.

We have demonstrated expression of membrane-associated human chorionic gonadotropin (hCG) by cultured human cancer cells of the nervous system and the rat C-6 glioma cell line. Monoclonal antibodies (MAbs) reacting with some hCG epitopes have shown cytolytic effects on human cancer cells. This study investigated whether intratumoral (IT) infusion of a MAb to hCGβ (CTP-103) would prolong survival of brain glioma-bearing rats. One million C-6 cells were infused via a chronic plastic guide cannula directed towards the striatum. The presence of intracerebral tumor was confirmed by magnetic resonance imaging (MRI) before submitting rats to infusion of either CTP-103 (12 μg/day) or PBS vehicle. Intratumoral infusion (0.5 μl/hr) was performed by means of a subcutaneous (sc) osmotic minipump connected to a 23g needle inserted through the guide cannula. A mean (± SEM) post C-6 cell implantation survival time of 23.6 ± 1.4 days with a range of 17-29 days was observed in a group of 8 non-treated C-6 cell-implanted rats. Rats infused with PBS IT or with CTP-103 sc died in 24 days. In contrast, 3 out of 4 IT CTP-103 treated rats are currently alive at 42, 88, and 91 days. Post-treatment MRI revealed the absence of tumor and the presence of necrosis and edema. Histological examination of an IT CTP-103 infused rat euthanized 78 days following tumor cell implantation confirmed the absence of tumor which had been observed by MRI prior to the start of treatment. These preliminary findings suggest that therapy for brain gliomas based on hCG MAbs may be effective. Supported by grants from Allegheny-Singer Research Institute, InVitro Technologies, Inc., the Silberman Family Fund of The Pittsburgh Foundation, and contribution in memory of B.D. Cadwallader.

839.1

CONCENTRATION DEPENDENT EFFECTS OF NEOSTIGMINE ON d-AMPHETAMINE INDUCED INCREASES IN STRIATAL ACETYLCHOLINE RELEASE. E. Acquas* and H. C. Fibiger. Division of Neurological Sciences, Dept. of Psychiatry, University of British Columbia, Vancouver, B. C., V6T 1Z3 Canada.

There is considerable evidence based on *in vivo* brain microdialysis that systemically administred d-amphetamine increases acetylcholine (ACh) release in the striatum. This increase is mediated by dopamine D_1 receptors, since it can be blocked by the D_1 receptor antagonists SCH 23390 and SCH 39166. In contrast, when d-amphetamine is applied directly to the striatum it decreases ACh release by its dopamine mediated actions on striatal D_2 receptors. Here, we report that the effects of d-amphetamine (2 and 10 mg/kg) on striatal ACh release are strongly influenced by the concentration of a cholinesterase inhibitor (neostigmine bromide) in the perfusion fluid. In the presence of neostigmine 10^{-7} M, d-amphetamine (2 mg/kg) significantly stimulated striatal ACh release (max increase 54% above baseline), while at 10^{-8} M this dose of d-amphetamine did not significantly change striatal ACh output (max 16% above baseline). Administration of a higher dose of d-amphetamine (10 mg/kg) significantly increased striatal ACh output in the presence of neostigmine 10^{-7} M (max 68% above baseline), while it decreased striatal ACh release (max 27% below the baseline) in the presence of 10^{-8} M neostigmine. In contrast to the neostigmine concentration dependent effects of d-amphetamine, enhanced ACh release produced by the full D_1 agonist A-77636 (4 μmol/kg) was not influenced by the level of cholinesterase inhibition. These findings indicate that the effects of d-amphetamine on striatal ACh release depend critically on the concentration of neostigmine in the perfusion fluid and demonstrate that microdialysis conditions can critically influence dopamine-mediated effects on striatal ACh release.

839.2

IN VIVO AND IN VITRO ACTIONS OF LINOPIRDINE AND NOVEL NEUROTRANSMITTER RELEASE ENHANCING AGENTS L.O. Wilkinson,*T. Smith, A. Ramirez, P. Seymour, S. McCarthy, L. Chambers, K. Ward, S. DeMattos, D. Liston, J. Lazzaro, A. Ganong, J. Lee, T. Schaeffer, D. Johnson, B. Volkmann, A. Villalobos, S.D. Heck, D.M. Nason, M.H. Lee, K. Coffman, G.M. Bright, J. Jasys, and W. F. White. Pfizer Central Research, Eastern Point Road, Groton, CT 06360

Linopirdine (DuP-996) is the first example of a novel class of compounds which increase the stimulated release of neurotransmitters in brain slices. Its ability to influence acetylcholine suggests utility in the treatment of Alzheimer's disease. We report here on the in vivo and in vitro activity of a number of succinimide and alcohol based compounds which, like DuP-996, modulate stimulated neurotransmitter release. As previously demonstrated, DuP-996 increased K+ stimulated release of acetylcholine from the striatum, displaying an inverted-U-shaped dose-response curve. In our succinimide and alcohol based series, a U-shaped dose-response curve was also observed. Structural modifications influenced maximal efficacy, but not potency. A comparison of the ability of these compounds to influence ACh release in the striatum and hippocampus revealed smaller effects in hippocampus than striatum, but the rank order of efficacy correlated in the two brain regions. To evaluate in vivo activity, ACh release from hippocampus was measured using the dialysis technique. Activity in this model correlated with in vitro efficacy and CSF drug concentration. These neurotransmitter release enhancers also produced a constellation of symptoms akin to those seen after treatments which increase cholinergic neurotransmission (fasiculations, salivation, tremors and seizures), and potentiated the ability of a pentylenetetrazol challenge to induce seizures in mice. The effects in all three of these in vivo models occurred in the same dose range; the rank order of potency of 7 compounds was the same in all three models. The close correlation between the effects of these compounds on ACh release in vivo and their ability to induce undesirable side-effects suggests that they occur through a similar mechanism.

839.3

IN VIVO MODULATION OF RAT CORTICAL ACETYLCHOLINE RELEASE BY NMDA RECEPTORS. M.G. Giovannini, L. Giovannelli, P. Blandina* and G. Pepeu. Dipartimento di Farmacologia, Università di Firenze, Firenze, Italy.

The transversal microdialysis technique was used to investigate the modulation of cortical cholinergic neurons by glutamatergic inputs in the rat *in vivo*. Male albino Wistar rats (250-300 g) were used. Under chloral hydrate anesthesia a transversal microdialysis membrane was positioned in the parietal cortex. Drugs were administered either through the dialysis membrane or i.c.v. The competitive NMDA antagonist CPP (5 nmol/5 µl saline) administered i.c.v. to rats elicited a long lasting increase in cortical ACh output by100%. This effect was not abolished by administration of NMDA (200 µM) to the cortex through the membrane. In some experiments a second membrane was inserted either in the medial septum or in the nucleus basalis magnocellularis (NBM). CPP (100 µM), locally administered to NBM, decreased cortical ACh release. CPP (100 µM) administered to the septum increased cortical ACh release by 82%, this effect being abolished by concomitant administration of 50 µM muscimol, while bicuculline alone (50 µM) increased cortical ACh release by 190%. These data indicate that cortical ACh release is indirectly modulated by a glutamatergic input. Retrograde tracing using Fluoro-Gold administered to the cortex *via* the dialysis membrane showed the presence of numerous labelled neurons in the nucleus basalis, an area known to project to the cortex, as well as sparse fluorescent cell bodies in the medial septal nuclei. Double labelling of the sections with an anti-ChAT antibody showed that the highly fluorescent neurons present in the medial septal nuclei and projecting to the cortex are not cholinergic. Further experiments using an anti-parvalbumin antibody will be performed to verify the nature, possibly GABAergic, of these neurons which might be responsible for the indirect modulation of cortical ACh release by glutamatergic inputs.

This project was supported by a Grant from MURST.

839.4

IS BUTYRYLCHOLINESTERASE PRESENT IN PRIMITIVE CHORDATES? L. Pezzementi*, R. Jotani, B. Mathews, D. Milner and W. Soong. Div. of Science and Mathematics, Birmingham-Southern College, Birmingham, Alabama, 35254.

Previously, we reported that a single cholinesterase (ChE), with properties intermediate to acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), was present in the cephalochordate amphioxus in globular and asymmetric forms. Sequence analysis of a PCR-generated 1kb genomic fragment was consistent with this identity (*Soc. Neurosci. Abstr.* 19, p. 917, 1993). Now, we find that two ChE activities can be distinguished kinetically by the irreversible inhibitor Iso-OMPA. A rapidly-inactivated fraction comprises 70% of the esterase in total high ionic strength detergent-containing extracts and resembles AChE, hydrolyzing acetylthiocholine almost exclusively. The residual slowly-inactivated enzyme resembles atypical BuChE found in cartilaginous and bony fishes, hydrolyzing butyrylthiocholine at 30% of the rate of acetylthiocholine. Most diagnostic ChE inhibitors do not distinguish the two esterases. The two activities are solubilized in different proportions by sequential extraction in low ionic strength (80% AChE/20% BuChE), low ionic strength detergent-containing (40%/60%), and high ionic strength detergent-containing buffers (70%/30%). We are determining the molecular forms of the enzymes. We have also used PCR with degenerate oligonucleotide primers to obtain a 1.5 kb ChE-like genomic sequence from amphioxus, which we are currently sequencing. In the agnathan vertebrate *Myxine glutinosa* we have found that globular and asymmetric forms of AChE are present in skeletal muscle, but that liver contains atypical BuChE; we have not yet characterized its molecular forms. Of the diagnostic inhibitors tested, only ±ambuterol distinguishes the two activities. In contrast, we have found only globular forms of AChE in the urochordate *Styela plicata*. These data require a reevaluation of the evolution of chordate AChE and BuChE. Supported by an NSF RUI grant to L.P.

839.5

REGULATION OF THE CHOLINERGIC GENE LOCUS BY RETINOIC ACID RECEPTOR ALPHA, cAMP, AND CNTF/LIF SIGNALING PATHWAYS IN A MURINE SEPTAL CELL LINE.
B. Berse[1], S.L. Wertheim[2]* and J.K. Blusztajn[1], [1]Boston University School of Medicine, Boston, MA 02118, and [2]University of Massachusetts Medical School, Worcester, MA 01655.

The genes encoding two proteins critical for the cholinergic phenotype: choline acetyltransferase (ChAT) and the vesicular acetylcholine transporter (VAChT) are closely linked - with the VAChT coding sequence contained within the first intron of the ChAT gene. By means of differential promoter use and alternative RNA splicing, the two genes generate two distinct classes of mRNA. This unusual genomic organization suggests that transcription of these two genes is co-ordinately regulated. Using Northern analysis and molecular probes specific for ChAT and VAChT, we have been studying the modulation of their expression in a murine septal cell line, SN56, by three groups of agents: retinoids, growth factors of the CNTF/LIF family, and cAMP. Messenger RNA levels of both ChAT- and VAChT were increased up to 3.5-fold by treatment with 9-cis- or all-trans-retinoic acid (RA, 1 µM, 48 h). This effect was mimicked by an RA receptor alpha (RARα) agonist (Ro 40-6055) and prevented by a specific antagonist (Ro 41-5253), indicating that it was mediated by RARα. ChAT- and VAChT-specific transcripts were also induced (up to 3-fold) by treatment with CNTF or LIF (20 ng/ml, 48 h), as well as by increasing intracellular [cAMP] (e.g. with 10 µM forskolin, which activates adenylate cyclase, or with 1 mM dibutyryl cAMP for 48 h). cAMP had a greater effect on the level of VAChT mRNA (4-fold induction) than on the level of ChAT mRNA (2-fold induction), suggesting a quantitatively differential transcriptional regulation of the two genes by the cAMP pathway. The effects of the three groups of agents studied were additive, pointing to several independent mechanisms by which the cholinergic properties of septal neurons can be modulated. (Supported by AG09525)

839.6

REGULATION OF CHOLINERGIC FUNCTION IN *CAENORHABDITIS ELEGANS*. D. Frisby, J. Duerr*, J. McManus, and J. Rand, Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

The *cha-1* and *unc-17* genes in *Caenorhabditis elegans* encode choline acetyltransferase and a vesicular acetylcholine transporter respectively. These genes were shown in our laboratory to comprise a eukaryotic "operon"; the *unc-17* gene is completely nested within the first intron of *cha-1*, and their cognate mRNAs are produced by alternative splicing of a common transcription unit. Analysis of the upstream region of *cha-1/unc-17* suggests the existence of a regulatory element (located within a 747 bp region approximately 2.4 kb upstream of exon-1) that is required for proper expression in most, but not all of the body cholinergic neurons (i.e., posterior to the pharynx). Expression of both genes in cholinergic neurons anterior to the nerve ring, however, does not appear to be altered in the absence of this putative "body control region." The upstream position of this region is consistent with the location of three independently isolated regulatory mutations which result in a phenotype similar to a deletion of the "body control region."

Supported by grants from NIH and OCAST

839.7

ELECTRICAL STIMULATION OF THE DORSAL RAPHE NUCLEUS INCREASES ACETYLCHOLINE RELEASE IN RAT FRONTAL CORTEX: AN *IN VIVO* MICRODIALYSIS STUDY.

H. Hirano* and H.C. Fibiger. Division of Neurological Sciences, Department of Psychiatry, The University of British Columbia, Vancouver, B.C., Canada V6T 1Z3

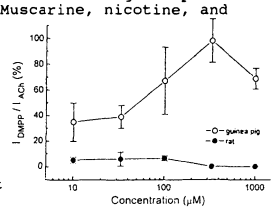
The effects of electrical stimulation (ES: frequency=60 Hz, stimulation duration=0.2 sec, interstimulus interval=2.1 sec) of the dorsal raphe nucleus (DRN) on cortically-projecting cholinergic neurons were studied using *in vivo* microdialysis to measure extracellular acetylcholine (ACh) in the frontal cortex of freely moving rats.

ES of the DRN for 20 min significantly increased cortical ACh concentrations in a current-dependent manner. Thus, ES at both 25 μA and at 50 μA significantly increased extracellular ACh levels, while ES at 12.5 μA failed to do so. Pretreatment with ketanserin, a 5-HT_{2A} receptor antagonist, failed to block ES (25 μA)-induced increases in cortical ACh release. Similarly, pretreatment with the selective dopamine D_1 antagonist SCH 23390 also failed to block the ES (25 μA)-induced increases in cortical ACh release. These results indicate that ES of DRN enhances cortical cholinergic function. However, neither 5-HT_{2A} nor D_1 receptors appear to be involved in this phenomenon.

839.8

PHARMACOLOGICAL DIFFERENCES IN THE CHOLINERGIC RECEPTOR ON OUTER HAIR CELLS OF RAT AND GUINEA PIG. C.Chen*, C. LeBlanc and R.P.Bobbin. Dept. of Otolaryngology, LSUMC, New Orleans, LA 70112.

The cholinergic receptor on outer hair cells (OHCs) in guinea pig has an unusual nicotinic-like receptor pharmacology (Erostegui et al., Hear. Res. 74: 135, 1994). The pharmacology of the acetylcholine (ACh) receptor cloned from rat cochlea (α_9 subunit) approximates but does not match the pharmacology obtained for the ACh receptor in guinea pig OHCs (Elgoyhen et al., Cell, 79: 705, 1994). The purpose of the present study was to compare cholinergic receptor agonist-induced responses in OHCs from rat to OHCs from guinea pig using the whole-cell variant of the patch-clamp technique. ACh caused the largest outward current in both groups of the cells recorded at 0 mV when stepped from a holding potential of -60 mV. Carbachol- and suberyldicholine-induced responses in cells from guinea pig were similar to those from rat. However, 1,1-dimethyl-4-phenylpiperazinium (DMPP) produced very little response in rat OHCs and a large response in guinea pig OHCs (see Fig.). Muscarine, nicotine, and cytisine induced very little response in guinea pig OHCs and none in rat OHCs. The pharmacology of the ACh receptor on OHCs of rat is similar to that obtained with the α_9 subunit in Xenopus oocytes, but different from the ACh receptor on OHCs of guinea pig.



Supported by an NIH grant R01-DC00722.

839.9

THE EFFECT OF AF64A ON CHOLINE ACETYLTRANSFERASE (ChAT) GENE EXPRESSION IN THE SEPTO-HIPPOCAMPAL PATHWAY IN VIVO.

Q. Fan, L.R. Santiago and I. Hanin*Dept. of Pharmacology, Loyola Univ. Chicago, Stritch School of Medicine, Maywood, Il 60153.

AF64A, a selective cholinergic neurotoxin, has been used to produce an animal model of cholinergic hypofunction (El Tamer et al., Neuropharmacology 31:397-402, 1992). We have, therefore, further focused our studies on the effect of AF64A on ChAT in the septo-hippocampal cholinergic pathway. Following a single administration of AF64A (i.c.v., bilaterally, 1 nmol/lateral ventricle), there was a transient increase in ChAT activity in the septum. This increase peaked (+164%, $p<.01$) at day 7, and was back to control levels at day 14. Concurrently, there was a long-lasting ($\geq$ 28 days) decrease (-40%, $p<.01$) in ChAT activity in the hippocampus. To determine if this change in ChAT activity was due to an effect of AF64A on gene expression of the cholinergic neuron, we quantified ChAT mRNA levels using the reverse-transcription polymerase-chain reaction approach. Histone mRNA was used as an internal standard. In septum, a significant increase (+165%, $p<.05$) in ChAT mRNA levels was observed as early as 1-2 days after the administration of AF64A, followed by a significant decrease (-54%, $p<.05$) at day 7. This reduction in septal ChAT mRNA levels was still observed at day 28. These combined data suggest that the long term effect of AF64A on the septo-hippocampal cholinergic pathway may, at least in part, be due to an action of AF64A on gene expression in the cholinergic neuron.

839.10

CHOLINESTERASE INHIBITORS INTERACTION WITH CORTICAL NEUROTRANSMITTERS. G. Cuadra and E. Giacobini*. Dept. Pharmacology, Southern Illinois Univ. Sch. Med., P.O. Box 19230, Springfield, IL 62794-9230 USA.

In previous investigations, we have demonstrated that cholinesterase inhibitors (ChEI) such as physostigmine (PHY) and heptylphysostigmine (HEP) elicit a significant and simultaneous increase in acetylcholine (ACh) and norepinephrine (NE) levels in rat cortex. This effect is enhanced by idazoxan (IDA), a selective α_2-antagonist. These data suggest that a combination of cholinergic and adrenergic drug may improve the pharmacological effect of ChEI on cortical neurotransmitters (NT) such as ACh-NE. In order to obtain additional information on cortical NT interaction, we evaluated, in the cerebral cortex of the rat, the effect of PHY and HEP in animals pretreated with clonidine (CLO), a selective α_2-agonist, on ACh, NE, dopamine (DA) and 5-hydroxytryptamine (5-HT, serotonin) extracellular levels. We detected no effect of systemic or intracortical CLO administration on ACh levels, but NE, DA and 5-HT levels were all decreased. Clonidine co-administration reduced the effect of PHY on ACh levels, on the contrary, HEP administered to animals pre-treated with CLO produced a stronger effect than HEP alone. A possible explanation for this difference is the variation in duration of the two drugs on ACh elevation and receptor desensitization. In conclusion, our data suggest that co-administration of a selective α_2-agonist such as CLO with ChEI does not represent a favorable pharmacological and therapeutical alternative. Considering our previous results with IDA (Cuadra and Giacobini, 1995), we suggest that combination of an α_2-antagonist with HEP may represent a more favorable alternative in order to improve the clinical efficacy of ChEIs in AD treatment.

839.11

CAFFEINE ENHANCES ACETYLCHOLINE RELEASE *IN VIVO* BY SELECTIVE ANTAGONISM OF ADENOSINE A_1 RECEPTORS. A.J. Carter*, W.T. O'Connor, M.J. Carter and U. Ungerstedt. Dept. of Biological Research, Boehringer Ingelheim KG, D-55126 Ingelheim/Rhein, Germany and Dept. of Physiology and Pharmacology, Karolinska Institute, S-17177 Stockholm, Sweden.

Caffeine is a commonly used drug which increases arousal, a condition associated with increased cholinergic activity in the mammalian cerebral cortex including the hippocampus. We have used the technique of microdialysis in association with microbore high-performance liquid chromatography to investigate the effects of caffeine on the extracellular levels of acetylcholine in the hippocampus of awake, freely moving rats. The oral administration of caffeine dose-dependently (3 - 30 mg/kg) increased the extracellular levels of acetylcholine. This increase was completely blocked when the microdialysis probe was perfused with the Na^+-channel blocker TTX, and strongly attenuated when a Ca^{2+}-free Ringer solution was employed. The effect of caffeine on hippocampal acetylcholine release was concentration-dependently counteracted by local perfusion of the A_1 receptor agonist N^6-cyclopentyladenosine (0.1 - 1 µmol/L), but not by the A_2 receptor agonist CGS 21680 (10 µmol/L). Neither agonist affected baseline acetylcholine release at these concentrations. These results demonstrate that acetylcholine release in the hippocampus is under tonic inhibitory control of the endogenous neuromodulator adenosine, and that orally administered caffeine enhances action potential-dependent vesicular acetylcholine release by antagonism of local A_1 receptors. Hence, the data provide a possible link between adenosine A_1 receptors in the hippocampus, increased cholinergic activity and the psychostimulant effects of caffeine.

839.12

MODULATION OF RAT CORTICAL ACETYLCHOLINE (ACh) RELEASE *IN VIVO* BY α2 ADRENOCEPTOR AGONISTS AND ANTAGONISTS. S. Tellez, F. Colpaert and M. Marien*, Centre de Recherche Pierre Fabre, Castres 81100, France.

Cortical ACh release in conscious guinea pigs was shown to be reduced by noradrenaline and clonidine (Moroni *et al.*, 1983, JPET 227, 435). The antagonism of this effect by yohimbine (a non-selective α2 adrenoceptor antagonist) suggested a mediation by α2 adrenoceptors. Recently we have shown that (+)-efaroxan, a highly selective α2 antagonist, increases cortical ACh release in the conscious rat, as measured by microdialysis (Eur. J. Pharmacol., in press). To further characterize this response, we have examined the effects of other α2 adrenoceptor ligands in the same model. The α2 antagonists (mg/kg), (+)-efaroxan (0.63), atipamezole (2.5) and idazoxan (2.5), increased cortical ACh outflow by 300, 230 and 220%, respectively, over a 3 h period following injection. In contrast, the α2 adrenoceptor agonist UK14,304 (2.5 mg/kg) decreased ACh outflow by 60%. The cholinesterase inhibitor tacrine had no significant effect on ACh outflow at 2.5 mg/kg i.p., increased outflow by 350-450% at 5 mg/kg, and was lethal at 10 mg/kg in 2 out of 3 rats. These results support the notion that cortical ACh release *in vivo* can be modulated by α2 adrenoceptors. Selective α2 adrenoceptor antagonists such as (+)-efaroxan may have a therapeutic potential in neurological disorders involving deficits in cortical ACh release.

839.13

DEPOLARIZATION-INDUCED BREAKDOWN OF CYTOSOLIC ACETYLCHOLINE IN RAT HIPPOCAMPAL NERVE TERMINALS: EFFECT ON ACETYLCHOLINE SYNTHESIS IN THE CYTOSOL. P.T. Carroll,* TTUHSC, Lubbock, Texas 79430.

Rat hippocampal minces were loaded with [acetyl 1-^{14}C] acetylcholine ([^{14}C] ACh) in the presence of echothiophate and the effect of high K^+ and veratridine depolarization determined on the subcellular storage and release of [^{14}C] ACh and its metabolites. Depolarization of tissue for 5 minutes released [^{14}C] acetate and decreased the ratio of [^{14}C] ACh to its metabolites in the cytosol (S_3) without stimulating the release of [^{14}C] ACh. When paraoxon was used instead of echothiophate, depolarization of tissue no longer released [^{14}C] acetate or decreased the ratio of [^{14}C] ACh to its metabolites in the S_3 fraction. Depolarization of tissue still did not release [^{14}C] ACh . However, when tissue was depolarized with veratridine for 10 minutes in the presence of echothiophate, [^{14}C] ACh was released. Also, the ratio of [^{14}C] ACh to [^{14}C] acetate in the S_3 fraction was reduced to the same extent as the ratio of [^{14}C] ACh to [^{14}C] acetyl CoA was increased, effects which were blocked by paraoxon. These results suggest that depolarization of hippocampal nerve terminals accelerates the breakdown of cytosolic ACh and thereby speeds up the "forward" reaction of cytosolic ChAT (BN581179758; NINDS 2R01N521289-10).

KEY WORD INDEX

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